THIS NUMBER COMPLETES VOL. VII

VOLUME VII NOVEMBER, 1924 NUMBER 6

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

Contents

A Study of the Relation Between Feed Consumption and Milk Secretion—C. W. TURNER 535

The Correlation Between Changes in Age and Milk Production of Dairy Cows under Other Than Official Testing Conditions—R. S. CLARK 547

Further Studies on the Bacterial Flora of the "Kingston Cheese"—C. D. KELLY 555

A Comparative Study of Methods for Determining Total Solids in Ice Cream—RICHARD C. FISHER AND CHARLES C. WALTS 576

The Use of True and Imitation Vanilla Extracts in Ice Cream—H. B. PIERCE, W. B. COMBS AND W. F. BORST 585

Index to Volume VII 591

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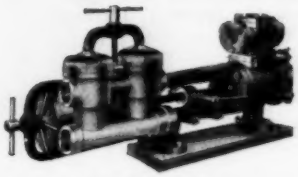

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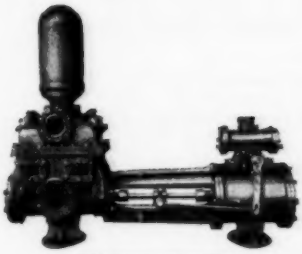

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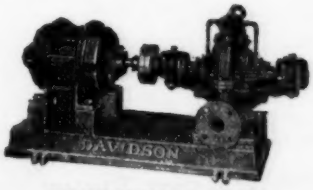

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Twenty-five reprints without covers of articles will be furnished gratis to contributors when ordered in advance. A table showing cost, with an order slip is sent with proof.

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Back Volumes: Volumes I–VI, incl., $36.00, United States, Canada, Mexico Cuba; $39.00, other countries. Single numbers, $1.25. Prices are net, postpaid

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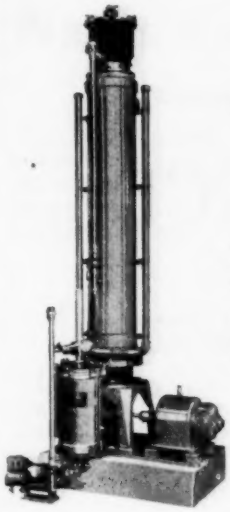

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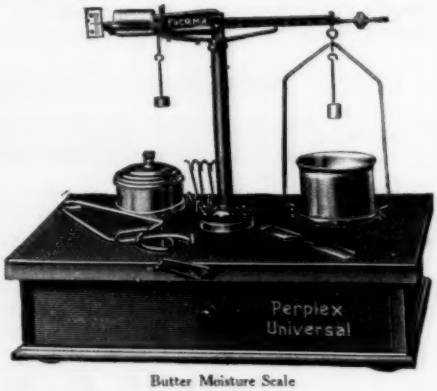

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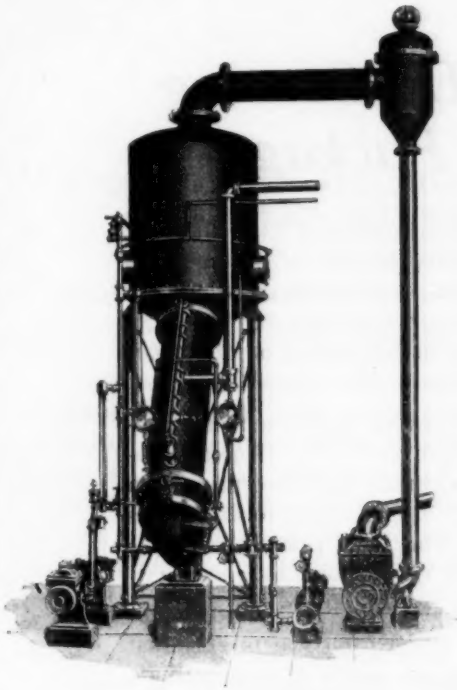

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A STUDY OF THE RELATION BETWEEN FEED CONSUMPTION AND MILK SECRETION

C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia, Missouri

Received for publication August 25, 1924

While many yearly milk production records are available which permit the study of the normal course of milk secretion during the lactation period, records of feed consumption during the course of the lactation period are very limited in number. Studies showing the relation between total feed consumption and total milk production are the basis of the modern feeding standards for dairy cattle, but the relation between feed consumption and milk secretion during the course of the lactation period has not been determined.

Study (1) of the characteristics of the yearly milk secretion curves of the various breeds of dairy cattle and a number of groups of cows within certain breeds indicate that under favorable conditions of feeding and management the factors controlling the rate of increase in milk secretion during the early part of the lactation period and the rate of decline following the period of maximum production appear to be inherited characteristics governing the mechanism of milk secretion.

The object of this paper is to present data showing the characteristics of the feed consumption curves during the course of the lactation period and their relation to milk secretion and change of live weight.

SOURCE OF DATA

Through the kindness of C. M. Long, Secretary of the Illinois Holstein Friesian Association and W. H. Dressel in charge of the Illinois Testing Plant at Dixon, Illinois, the writer secured for study and analysis the complete feed, milk, live weight, age,

and breeding records of approximately 50 Holstein-Friesian cows on yearly test. Grateful acknowledgment to these gentlemen for making this study possible is given.

FEED AND MANAGEMENT OF TEST COWS

The cows at the Illinois testing plant were provided with individual box stalls. As each cow was kept isolated so as to prevent spread of disease it was impossible to let the cows out to pasture. A limited amount of exercise, however, was provided. In many cases the cows were brought to the plant several months previous to the time of calving in order to be properly fitted for the test. While feed records were kept during this period they were not studied.

Complete accurate records were kept of the concentrates and roughage fed to each individual cow. The daily milk production was recorded as required for Advanced Registry. The weight of each cow was carefully estimated by the feeder and herdsman each month. The date of service was also recorded. In general it may be said that the cows were kept under the best possible conditions and every effort was made to secure maximum milk production from each cow.

The records should therefore be (as nearly as it is possible to secure) the actual inherited capacity for production. The course of milk secretion should also represent the inherited rate of increase in milk production, the amount of production at the maximum, and the rate of decline of milk secretion or the persistency of production.

As feed was provided up to the maximum of consumption without throwing the cow off feed, the feed records should indicate the rate of increase of feed consumption, the time of maximum consumption, and the rate of decline of the appetite as milk secretion declines.

FEEDS USED AT TESTING PLANT

In table 1 a list of all feeds used at the testing plant are included. The content of digestible nutrients in the several feeds were taken from the appendix table of "Feeds and Feeding"

by Henry and Morrison. The degestible nutrients in the commercial mixed feeds were secured from the manufacturer. This list includes most of the popular feeds fed to test cows in the mid-west.

TABLE 1

Feeds used at the testing plant

NAME OF FEED	CRUDE PROTEIN	CARBO-HYDRATE EQUIVALENT	TOTAL DIGESTIBLE NUTRIENTS
Ground corn (dent grade 2)	7.1	74.6	81.7
Corn germ meal	16.5	66.0	82.5
Corn gluten meal	30.2	53.8	84.0
Cottonseed meal	37.0	41.2	78.2
Distillers grains (from corn)	22.4	66.5	88.9
Hominy feed	7.0	77.6	84.6
Linseed meal (O. P.)	30.2	47.7	77.9
Oat meal (rolled oats)	12.8	70.4	83.2
Oats, ground	9.7	60.7	70.4
Soybean oil meal	39.7	44.8	84.5
Cocoanut meal (low in fat)	18.8	60.2	79.0
Wheat bran (all analyses)	12.5	48.4	60.9
Wheat middlings (shorts)	13.4	55.9	69.3
Alfalfa hay (all analyses)	10.6	41.0	51.6
Beet pulp	4.6	67.0	71.6
Beets, common	0.9	9.3	10.2
Corn silage	1.1	16.6	17.7
Unicorn	21.3	61.0	82.3
Purina cow chow	20.2	51.5	71.7
Champion (estimated)	20.2	54.3	74.5

RELATION BETWEEN THE INITIAL INCREASE IN FOOD CONSUMPTION AND MILK SECRETION

From the daily feed records, the amount of digestible crude protein, carbohydrate equivalent (fat $\times$ 2.25 + carbohydrate) and total digestible nutrients consumed by each cow was determined. The average daily consumption of digestible nutrients for the first sixty days is presented in table 2. In figure 1 is presented the relation between feed consumption and milk secretion during this period. It will be seen that there is a gradual increase in the nutrients received throughout the entire sixty-day period. In fact there is a slight increase until the

fourth month in the average daily consumption of nutrients. In comparison to feed consumption, milk secretion increases rapidly reaching a maximum about thirty-five days after parturi-

TABLE 2

Average daily feed consumption

DAYS AFTER CALVING	DIGESTIBLE CRUDE PROTEIN	CARBOHYDRATE EQUIVALENT	TOTAL DIGESTIBLE NUTRIENTS	DAYS AFTER CALVING	DIGESTIBLE CRUDE PROTEIN	CARBOHYDRATE EQUIVALENT	TOTAL DIGESTIBLE NUTRIENTS
	pounds	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
1	3.75	18.38	22.13	31	5.37	22.75	28.12
2	3.82	18.65	22.47	32	5.39	22.92	28.31
3	3.90	18.86	22.76	33	5.42	22.99	28.41
4	3.95	18.97	22.92	34	5.43	23.24	28.67
5	4.11	19.67	23.78	35	5.46	23.26	28.72
6	4.21	19.89	24.10	36	5.47	23.11	28.58
7	4.29	20.14	24.43	37	5.52	23.18	28.70
8	4.29	20.11	24.40	38	5.56	23.30	28.86
9	4.42	20.01	24.43	39	5.62	23.44	29.06
10	4.53	20.08	24.61	40	5.62	22.97	28.59
11	4.56	20.13	24.69	41	5.64	23.52	29.16
12	4.64	20.28	24.92	42	5.66	23.54	29.20
13	4.68	20.43	25.11	43	5.66	23.50	29.16
14	4.76	20.68	25.44	44	5.66	23.51	29.17
15	4.81	20.82	25.63	45	5.66	23.55	29.21
16	4.80	20.89	25.69	46	5.68	23.57	29.25
17	4.96	21.20	26.16	47	5.72	23.57	29.29
18	5.16	21.16	26.32	48	5.76	23.68	29.44
19	5.18	21.10	26.28	49	5.72	23.61	29.33
20	5.24	21.83	27.07	50	5.75	23.74	29.49
21	5.26	21.95	27.21	51	5.75	23.73	29.48
22	5.27	21.99	27.26	52	5.75	23.75	29.51
23	5.26	22.49	27.75	53	5.74	23.77	29.51
24	5.38	22.48	27.86	54	5.76	23.82	29.58
25	5.29	22.08	27.37	55	5.76	23.85	29.61
26	5.33	22.23	27.56	56	5.77	23.87	29.64
27	5.34	22.25	27.59	57	5.77	23.87	29.64
28	5.34	22.30	27.64	58	5.78	23.92	29.70
29	5.36	22.36	27.72	59	5.80	23.95	29.75
30	5.35	22.42	27.77	60	5.80	24.04	29.84

tion. This milk is evidently secreted, in the absence of a sufficient intake, at the expense of the body, causing a loss of live weight. It is evident from these facts that the initial rise in milk secretion may be limited by the condition of the cow

at the time of calving as well as by the frequency of milking (1) and the inherited factors which regulate or limit the rate of increase of secretion. It is important, therefore, in studying

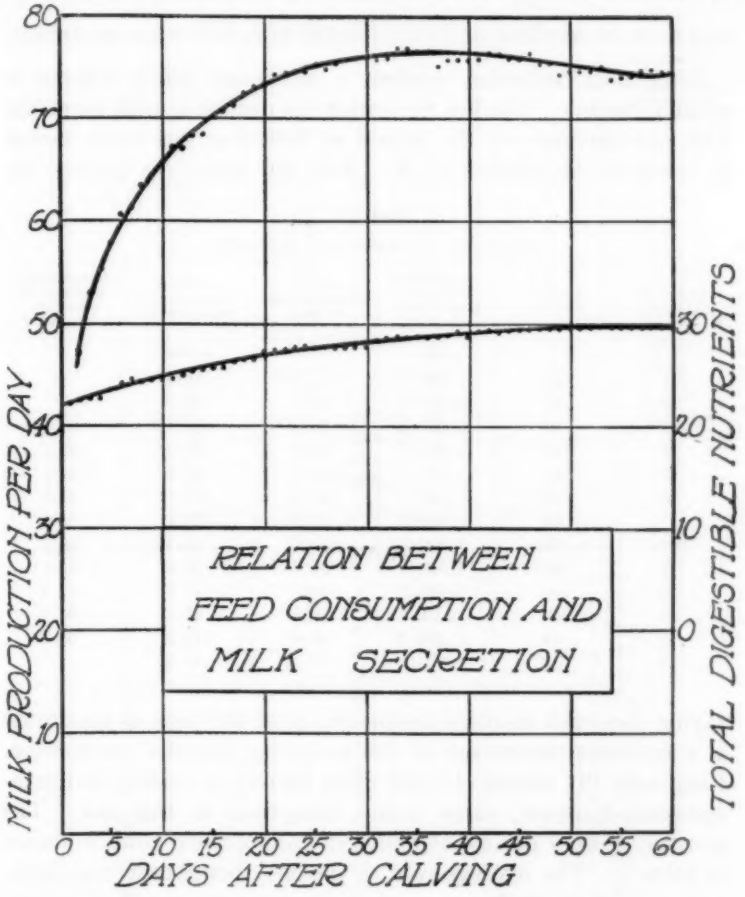

FIG. 1. RELATION BETWEEN FEED CONSUMPTION AND MILK SECRETION DURING THE FIRST SIXTY DAYS AFTER CALVING

While milk secretion increases rapidly reaching a maximum about thirty-five days after calving, there is a gradual increase in the nutrients consumed throughout the entire sixty-day period.

the initial rise of milk secretion, that the various factors which may limit or retard the full demonstration of the inherited qualities be understood and controlled as far as possible.

THE RATE OF DECLINE OF FOOD CONSUMPTION AND MILK SECRETION

After milk secretion reaches a maximum there follows a gradual decline. The law governing the decline of milk secretion with the advance of the period of lactation has been shown by work at this Station (1, 2). This law may be expressed by

TABLE 3

Summary of monthly milk production

MONTH OF LACTATION	NUMBER OF COWS	AVERAGE MONTHLY PRODUCTION OF MILK	AVERAGE NUMBER DAYS IN MILK	AVERAGE DAILY MILK PRODUCTION	PERCENTAGE OF PREVIOUS MONTH'S PRODUCTION
		pounds		*pounds*	
1	49	1,028.2	15.5	66.3	
2	49	2,436.1	30.2	80.7	
3	49	2,222.9	30.3	73.4	91.1
4	49	2,084.7	29.8	70.0	95.3
5	49	1,957.9	30.5	64.2	91.7
6	49	1,846.1	30.4	60.7	94.6
7	49	1,690.7	30.4	55.6	91.5
8	49	1,624.7	30.6	53.1	95.5
9	49	1,528.7	30.3	50.5	95.1
10	49	1,460.7	30.6	47.7	94.4
11	49	1,350.7	30.0	45.0	94.3
12	48	1,266.2	29.3	43.2	96.0
13	39	721.3	16.4	43.9	

saying that each month's production after the time of maximum is a constant percentage of the preceding months' production. Pregnancy (3), season (4), and other factors of feeding and management, however, cause minor exceptions to this law. The monthly rate of decline of milk secretion of these cows is shown in table 3. The average rate of decline thoughtout the lactation period is 93.95 per cent of the previous month's production. This factor indicates the persistency of the cows included.

How does the decline in feed consumption compare with the decline in milk secretion? As shown by table 4, feed consumption

also appears to follow a definite law of decline. It may be expressed as follows: After maximum feed consumption is

TABLE 4

Average daily feed consumption by months

MONTH OF LACTATION	DIGESTIBLE CRUDE PROTEIN	CARBOHYDRATE EQUIVALENT	TOTAL DIGESTIBLE NUTRIENTS	NUTRITIVE RATIO	PERCENTAGE PREVIOUS MONTH'S FEED CONSUMPTION
	pounds	*pounds*	*pounds*		
1	4.63	20.38	25.01	1:4.4	
2	5.41	22.76	28.17	4.2	
3	5.69	23.36	29.05	4.1	
4	5.83	24.05	29.88	4.1	
5	5.92	23.55	29.47	3.9	98.62
6	5.81	23.28	29.09	4.0	98.71
7	5.81	22.55	28.36	3.8	97.49
8	5.77	22.39	28.16	3.8	99.29
9	5.59	21.90	27.49	3.9	97.62
10	5.57	21.32	26.89	3.8	97.81
11	5.29	20.93	26.22	3.9	97.51
12	5.17	20.22	25.39	3.9	96.83

TABLE 5

Summary of monthly change in live weight

MONTH OF LACTATION	NUMBER OF COWS	AVERAGE LIVE WEIGHT
		pounds
1	45	1,370
2	46	1,321
3	46	1,296
4	46	1,300
5	46	1,304
6	46	1,315
7	46	1,314
8	46	1,311
9	46	1,319
10	46	1,338
11	46	1,356
12	45	1,384
13	43	1,443

reached, each month's food consumption is a constant percentage of the preceding month's food consumption. The rate of decline of the appetite, indicated by daily feed consumption is slower

than the rate of decline of milk secretion. The average rate of decline of feed consumption is 97.98 per cent of the previous month's consumption.

With a physiological condition prevailing in which the feed consumed contains an excess of nutrients needed for milk secretion, the surplus must be stored as an increase in body weight. This is in fact what happened as shown by table 5.

These data are therefore taken to indicate that under optimum conditions of feeding and management, the curve of milk secretion is regulated by certain inherited factors governing the initial rise of milk secretion, the volume of secretion at the maximum, and the persistence or rate of decline of milk secretion. An excess of feed is merely stored as an increase in body weight. The futility of over-feeding to increase milk secretion is thus shown.

Diary cows whose inheritance for milk production is great may be limited by the lack of capacity for feed consumption. In that event, the decline or persistency in milk production will parallel the decline of feed consumption and the animal will be in thin flesh at the close of the lactation period. Such an animal is said to have "dairy temperament." On the other hand, in animals whose inheritance for milk production or "persistency" is less, the capacity for feed consumption is greater than the need of nutrients for milk production and they will use the surplus for an increase in body weight.

The animals included in the study on the average consumed feed slightly in excess of their capacity for milk production. The limiting factor in their inheritance for milk production was in lacking persistency of milk production rather than a lack of capacity for the consumption of feed.

NUTRIENTS REQUIRED FOR MILK PRODUCTION

There has been considerable discussion among feeders of test cows as to the changes which should be made in the relation between the digestible protein and the carbohydrate equivalent (the nutritive ratio) during the lactation period. This relation

during the course of the lactation period for the cows studies is given in table 4. It will be noted that the nutritive ratio was decreased slightly during the first six months (from 1:4.4 to 3.9) and then was held fairly constant during the rest of the lactation period.

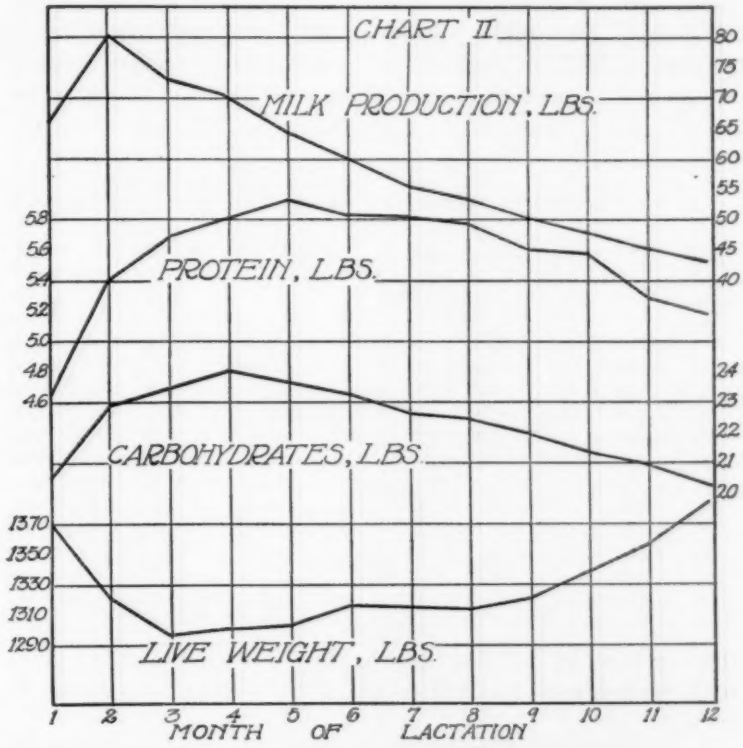

FIG. 2. THE CHANGE IN MILK PRODUCTION, FEED CONSUMPTION, AND LIVE WEIGHT DURING THE COURSE OF THE LACTATION PERIOD

In figure 2 the average daily milk production during the lactation period is compared with digestible protein and carbohydrate equivalent consumption. This shows the nutrients consumed to produce the various amounts of milk during the course of the lactation period.

By subtracting the requirements for maintenance prescribed by the Haecker Standard (0.7 pounds digestible crude protein and 7.925 pounds total digestible nutrints per 1000 pounds live weight) from the consumption of the various nutrients and dividing it by the average daily milk yield, the requirement per pound of milk during each month of lactation was determined for 3.5 per cent milk. To make the data applicable to cows producing milk of various fat content the Haecker feeding standard was used as a guide because it is based on extensive

TABLE 6

Feeding standard for cows on test

Month of lactation	Per cent fat 2.5		Per cent fat 3.5		Per cent fat 4.5		Per cent fat 5.5		Per cent fat 6.5	
	Protein	Carbohydrate equivalent	Protein	Carbohydrate equivalent	Protein	Carbohydrate equivalent	Protein	Carbohydrate equivalent	Protein	Carbohydrate equivalent
1	0.0508	0.126	0.0553	0.158	0.0643	0.189	0.0722	0.216	0.0813	0.245
2	0.0510	0.131	0.0555	0.164	0.0646	0.196	0.0725	0.224	0.0815	0.254
3	0.0598	0.152	0.0651	0.191	0.0757	0.228	0.0850	0.261	0.0957	0.296
4	0.0646	0.166	0.0703	0.209	0.0818	0.250	0.0918	0.285	0.1033	0.324
5	0.0715	0.175	0.0779	0.220	0.0906	0.263	0.1017	0.301	0.1145	0.341
6	0.0739	0.181	0.0805	0.227	0.0936	0.271	0.1051	0.310	0.1183	0.351
7	0.0807	0.187	0.0879	0.235	0.1022	0.281	0.1148	0.321	0.1292	0.364
8	0.0808	0.193	0.0880	0.243	0.1024	0.290	0.1149	0.332	0.1293	0.376
9	0.0849	0.195	0.0924	0.245	0.1075	0.293	0.1207	0.335	0.1358	0.379
10	0.0890	0.195	0.0969	0.245	0.1127	0.293	0.1266	0.335	0.1424	0.377
11	0.0890	0.197	0.0969	0.247	0.1127	0.295	0.1266	0.337	0.1424	0.382
12	0.0893	0.189	0.0972	0.237	0.1131	0.283	0.1270	0.324	0.1428	0.367

data showing the relation between the fat content of milk and its feed requirement for production. The results are shown in table 6.

It is generally believed that the feed cost of milk production under official test conditions is greater than the production under ordinary care. In other words, it is thought the economic law of diminishing returns begin to operative with test cows as milk production increases. Unquestionably this is true in those cases where cows are fed above their inherited capacity for milk production. But until that point is reached it was

found that the milk produced per pound of grain increased as the average yearly milk production increased. The results are shown in tables 7 and 8.

TABLE 7

Relation between yearly milk production and feed consumption

NUMBER OF COWS	MILK	FAT	GRAIN	HAY	BEET PULP	SILAGE
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
4	15,260	541	5,579	4,143	1,686	8,672
10	16,996	576	5,892	4,107	1,541	8,556
7	19,310	640	6,624	4,409	1,794	8,977
7	20,847	689	6,828	4,279	1,722	8,964
10	22,833	733	6,874	4,259	1,767	8,870
4	24,808	768	6,649	4,164	1,612	8,671
4	26,605	830	7,739	4,400	1,781	9,226

TABLE 8

Milk produced per pound of feed

NUMBER OF COWS	MILK	FAT	GRAIN	HAY	BEET PULP	SILAGE
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
4	15,260	541	2.73	3.70	9.04	1.75
10	16,995	576	2.88	4.13	11.03	1.98
7	19,310	640	2.91	4.37	10.70	2.15
7	20,487	689	3.05	4.87	12.00	2.32
10	22,833	733	3.32	5.36	12.90	2.57
4	24,808	768	3.69	5.90	15.20	2.83
4	26,605	830	3.43	6.04	14.90	2.88

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THE CORRELATION BETWEEN CHANGES IN AGE AND MILK PRODUCTION OF DAIRY COWS UNDER OTHER THAN OFFICIAL TESTING CONDITIONS[1]

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Received for publication September 17, 1924

Previous investigations have shown the correlation that exists between the changes in age and milk production of dairy cows when the cows are kept under official testing conditions. Many cattle breeders have wondered whether or not the results of those investigations could be applied with accuracy to conditions such as those under which a cow is not being fed and managed for an official record.

It is important that the truth be known concerning the correlation of changes in age and milk production of cows kept under other than official test conditions, since a very small percentage of the dairy cattle of the country are tested for official record. For these cows without official test, there is a need for age correction factors, such that can be used with the immature record of a young cow so as to predict the probable mature production of that animal. With the use of such factors the breeders can tell how profitable the young cow will be when she becomes mature, with at least a fair degree of accuracy.

In order to determine age correction factors for other than official test conditions, the author has studied records from the experiment station herds of the following colleges and universities: University of Minnesota (1), Cornell University (2), University of Nebraska (3), University of Vermont (4), Storrs Connecticut Station (5), University of Missouri (6), Oregon Agricultural

[1] The material for this article is taken from work preliminary to a thesis by R. S. Clark, for the degree of Master of Science at the University of Minnesota.

College (7), University of Wisconsin (8), University of Illinois (9), University of Maryland (10), and the Pennsylvania State College (11).

METHODS OF HANDLING DATA

In collecting the data from which the age correction factors were to be calculated, all irregularities were noted in so far as the records available showed. No records immediately following abortions were used.

The age at which a cow freshened was calculated from the date of birth to the date on which the calf was born. The record was tabulated as of the age to which it was closest. The considerable number of records used should eliminate the error that would result from placing some records under a certain age merely because of the margin of a few days.

No records were included in the age correction study from cows having less than two yearly records. None were used that were made with more than two milkings daily in so far as the records showed. The season at which a cow freshens affect somewhat the percentage of fat in the milk and it may have some effect on the quantity as well.

No correction factors have been calculated by previous workers to equalize this influence and this investigation does not include a determination of such factors. It would be difficult to work out factors for the influence of season of freshening. It would also be hard to apply those factors. A factor for each day would need to be determined so as to cover every possible date of freshening.

The length of gestation period during the time of milking affects milk production. The herds studied were handled in a manner representative of good herd management. The numbers involved should avoid any considerable error on this point. In cases where a cow milked for more than a year in one lactation period, the record for the first 365 days only, was used.

While no information is at hand regarding the details of the feeding practice in the several herds supplying data, it is believed

that these conditions are reasonably uniform and that no special errors are introduced by such differences as do exist.

To measure the mathematical reliability of the data, the standard deviations, coefficients of variability and their probable errors were calculated. Standard formulae were used in these calculations (12).

The data for each of the four breeds studied were tabulated separately. Table 1 shows the division of the material among the Holstein, Jersey, Guernsey, and Ayrshire Breeds.

TABLE 1

Summary of records

Age correction factors to tenth year*

BREED	NUMBER OF RECORDS	NUMBER OF COWS
Holstein	280	77
Jersey	632	189
Guernsey	540	139
Ayrshire	231	70

* The number of records in the ages beyond ten years was so limited in most breeds that those ages were merely tabulated and were not given statistical consideration. Ten years was a satisfactory age at which to conclude the analysis since the point of mature production was found to occur prior to that age for every breed.

DISCUSSION OF DATA

Chart 1 shows in a visual way the results of the investigation. The calculated curve was obtained by the use of the "Star Point Method" (13). The formula for this method is,

$$y = x \text{ plus } bx \text{ plus } cx^2 \text{ plus } dx^3 \ldots \text{ etc.}$$

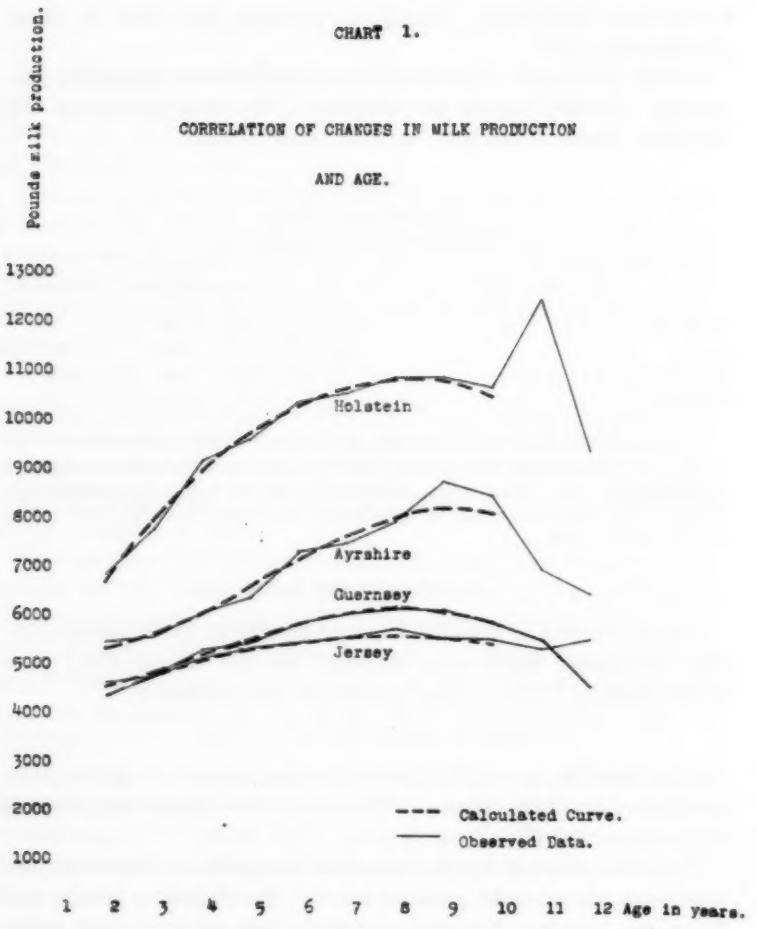
In this formula, $y =$ milk yield in pounds and $x =$ age in years as applied to these figures. The chart also shows the plotting of the observed data.

From the chart it can be seen that the point of maximum production is about eight years of age for the Holstein, Jersey, and Guernsey breeds. For the Ayrshires this point is nine years.

Even with experiment station herds there was some discarding of low producers after one or two records have been placed on

CHART 1.

CORRELATION OF CHANGES IN MILK PRODUCTION AND AGE.



each cow. Allowance must be made for this fact when using these figures. There is however, much less selection of the population in the experiment station herds, than in the case of Advanced Registry Cows.

Table 2 includes the figures from which the observed and calculated curves were charted.

In table 3, a comparison is made between the maturity represented by the ages studied in this investigation and the results

TABLE 2

Age correction data

Calculated and observed data arranged by breeds

CLASS MID POINTS	HOLSTEIN			JERSEY			GUERNSEY			AYRSHIRE		
	Number of cows	Milk		Number of cows	Milk		Number of cows	Milk		Number of cows	Milk	
		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated
years		*pounds*	*pounds*		*pounds*	*pounds*		*pounds*	*pounds*		*pounds*	*pounds*
2	35	7047.7	6881.1	62	4591.7	4619.0	102	4837.2	4750.0	22	5666.3	5510.0
3	50	7949.0	8110.6	93	4998.5	4962.5	110	4996.6	5050.5	41	5774.4	5821.3
4	52	9311.6	9106.9	84	5342.9	5268.6	83	5500.1	5391.4	45	6287.1	6276.8
5	42	9767.2	9900.0	86	5555.9	5537.4	69	5688.1	5700.0	36	6595.0	6800.0
6	29	10530.5	10479.9	78	5693.2	5696.8	53	6066.3	6052.4	23	7526.6	7354.4
7	24	10722.5	10846.6	77	5770.1	5783.0	46	6281.4	6280.7	25	7702.9	7843.2
8	22	11092.1	11000.1	58	5908.6	5795.8	35	6346.7	6400.0	16	8156.6	8200.0
9	17	11098.6	10940.4	54	5756.9	5745.3	24	6313.4	6278.0	12	8968.1	8387.6
10	9	10894.1	10667.5	40	5717.0	5601.5	18	6083.4	6100.0	11	8688.1	8300.0
11	9	12691.1		38	5513.0		14	5742.8		7	7185.4	
12	7	9559.2		19	5748.5		8	4759.1		7	6659.1	
13	6	10262.1		13	6032.7		4	5744.3		4	6406.1	
14	2	11583.3		12	5818.7		3	5199.4		4	6493.6	
15	1	11547.7		3	6453.4		1	6041.1		4	5977.2	
16	1	8119.1		2	6870.3		1	3286.3		1	4685.0	

as secured by other workers. The percentages given by Gowen and Ragsdale, as referred to in the footnote on the table, represent yearly butterfat records of official testing. Those of Pearl and Miner are from the Scottish Ayrshire records, and represent yearly equivalents of butter-fat. The breed association advanced register requirements were used for calculating the percentages given in the table as coming from this source. The butterfat requirements were the basis of calculation.

TABLE 3

Relation of age to milk production expressed in percentage

Comparison made between several sources

BREED	2 YEARS	3 YEARS	4 YEARS	5 YEARS	6 YEARS	7 YEARS	8 YEARS	9 YEARS	10 YEARS	11 YEARS	SOURCE OF DATA
Holstein	66.2	73.7	82.8	90.0	95.3	98.6	100.0	95.5	97.0		College herds*
	68.2	77.6	84.1	94.5	96.3	99.9	100.0				Gowen†
	73.0	83.9	87.1	90.6	96.1	96.6	100.0	95.6	93.5		Ragsdale et al.‡
	66.3	77.5	88.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Association§
											Requirements for Advanced Registry record
Jersey	79.7	85.8	90.9	95.5	98.3	99.3	100.0	99.1	96.6		College Herds*
	67.4	79.2	87.1	92.7	97.2	99.4	100.0				Pearl et al.¶
	73.1	84.4	92.7	95.8	98.5	99.4	100.0	97.3	97.1		Ragsdale et al.‡
	72.6	81.8	90.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Association§
											Requirements for Advanced Registry record
Guernsey	74.2	78.9	84.2	89.1	94.6	98.1	100.0	98.1	95.3		College Herds*
	64.4	80.3	87.1	92.1	95.9	98.3	99.7	100.0			Gowen**
	78.0	85.6	93.2	96.8	98.5	100.0	100.0	99.2	96.9		Ragsdale et al‡
	72.6	81.8	90.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Association§
											Requirements for Advanced Registry record
Ayrshire	65.7	69.4	74.8	81.1	87.7	93.5	97.7	100.0	98.9		College Herds*
	70.2	78.7	84.7	88.9	92.3	95.0	97.1	98.6	99.5	100.0	Pearl and Miner††
	74.2	81.4	87.3	92.9	97.5	99.6	100.0	98.1	96.2		Ragsdale et al.‡
	66.3	77.5	88.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Associaton§
											Requirements for Advanced Registry record

* These data were secured from experiment station herds. See text, page 547.

† M. S. and J. W. Gowen, Maine Bul. 306, p. 27.

‡ Arthur C. Ragsdale, et al., Jour. of Dairy Science, vii, no. 2, p. 191.

§ Requirements for entry into Advanced Registry as given by breed associations in America. See text, p. 551.

¶ R. Pearl, et al., Maine Bul. 281, p. 92.

** J. W. Gowen, Maine Bul. 311, p. 14–15.

†† R. Pearl and J. R. Miner, Jour. Agr. Res., xvii, p. 285–320.

For the Holstein breed the requirements of the 365-day division were used. The 365-day division of the single letter class of the Jersey register of merit furnished the percentage for this breed. Also the single letter class of the Guernsey requirements was used. For the Ayrshire figure the 365-day fat requirements as adopted in June, 1924, were used.

The use of fat requirements and *fat* production records in comparison with the records of *milk* production of these ages is justified since Gowen (14) has found only slight correlation between changes of age and fat percentage (-0.0546 plus or minus 0.0181). Furthermore, Eckles (15) studied official and non-official records of considerable number and concluded, that "The figures show that the richness of the milk remains practically constant, fluctuating slightly with individuals from year to year, until the animal is past her prime, when it slowly declines with advancing age."

The percentages of table 3 may be used to predict the probable mature production of a young cow when that cow has already made one or more records under non-official test conditions and under conditions that represent good herd management. The calculation involved is division of the record by the percentage of the breed and age represented as given in Table 3. The quotient is multiplied by 100 to get the predicted mature production. Thus a two-year old Ayrshire giving 6570 pounds of milk may be expected to produce $(6570 \div 65.7) \times 100 = 10{,}000$ pounds of milk, when she is mature.

Greater accuracy of prediction will result when there are available two or more records on the cow at immature ages. In such a case each record can be calculated to maturity and the average will represent more nearly, the probable mature production than when only one record is used.

SUMMARY

1. One thousand six hundred eighty-three yearly records from 475 cows are included in a study of the relation between changes in age and milk production of dairy cows. The records were made in eleven experiment station herds in the United States.

2. The Holstein, Jersey, Guernsey and Ayrshire breeds are studied separately.

3. Only records made under other than official test conditions are used.

4. The age of maximum milk production is found to be eight years for the Holstein, Jersey, and Guernsey breeds and for the Ayrshire, nine years, under the conditions studied.

5. The investigation shows that the increase in production is small after six years of age except in the case of Ayrshires which reach maturity one year later than the other three breeds.

6. A table is given comparing the percentages of maturity of milk production at the different ages of this investigation with the percentages as shown by records made under other conditions. The chart included the percentages as derived from the advanced register and register of merit requirements of the several breed associations.

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FURTHER STUDIES ON THE BACTERIAL FLORA OF THE "KINGSTON CHEESE"[1]

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Received for publication June 10, 1924

INTRODUCTION

The process of manufacture of "Kingston cheese" was worked out in England by Alec. Todd and Wilfrid Sadler (15) to meet the demand for a cheese of the hard pressed variety, a cheese not too large for the average family and one which could be used with little or no waste. While this cheese is made on the hard pressed principle, the system and procedures employed are such as to give a cheese that will ripen in ten days. The ripe cheese is usually 1 pound in weight, has a flavor of its own and a soft granular texture, rich and buttery. For the past three years this cheese has been manufactured in the laboratories of the University of British Columbia. In a thesis presented by me in April, 1922 (8), detailed references were made to the system on which the cheese is manufactured, and complete records of the cheese made during a large part of the year were presented. The thesis included, moreover, the results of bacteriological examinations of certain of the cheese referred to immediately above. As far as the bacteriological examinations were concerned, the results were very incomplete, yet the technique worked out at that time, and the data obtained, have served as an invaluable preliminary to carrying on the work reported herein.

[1] This paper was presented in partial fulfilment of the requirements for the Degree of Master of Science in Agriculture in the University of British Columbia, May, 1924.

The work was so planned that quantitative bacteriological analyses were to be made of a cheese when one day old, and of a cheese of the same day's make when ready for market, also the organisms occurring with the greatest frequency were to be isolated from the plates of each cheese examined. It has been anticipated that the data thus secured might lead to some information being obtained on the ripening processes of this cheese.

BACTERIOLOGICAL EXAMINATIONS OF THE "KINGSTON CHEESE"

MEDIA EMPLOYED

Beef-peptone-agar, Standard Methods (12).

Glucose-agar 0.5 per cent glucose added to beef-peptone-agar.

Lactose-agar 0.5 per cent lactose added to beef-peptone-agar.

Milk-agar, Ayers and Mudge (1).

MacConkey's neutral-red-bile-salt-lactose-agar (11) for specific reactions of the organisms which ferment lactose to acid and gas.

MacConkey's neutral-red-bile-salt-lactose-broth (11) for detecting the presence of the organisms which ferment lactose to acid and gas.

Nutrient-agar to which specific carbohydrates were added as desired, using brom-cresol-purple as indicator (3) (14).

Nutrient-broth to which specific carbohydrates were added as desired, using brom-thymol-blue as indicator (12).

Peptone-medium for Methyl Red and Voges-Proskauer determinations. Standard Methods (12).

Peptone-medium for Indol Production. Standard Methods (12).

Nitrate-agar. Manual of Methods (14).

Glucose-agar and glucose-gelatin were used throughout for the quantitative plate counts. For comparative purposes lactose-agar and milk-agar (1) were used in addition to the two media mentioned above. The dilution method of quantitative analyses was employed when using litmus-milk and the Voges-Proskauer broth.

Reaction of media

All media, excepting bile-salt media, were adjusted according to hydrogen-ion concentration. The final reaction was pH = 7 using brom-thymol-blue as indicator (12).

Examination and technique

The same technique was used for the bacteriological examinations of the cheese as was worked out during the previous studies on the "Kingston cheese" (8). On arrival at the laboratory the one pound cheese was cut in quarters and the necessary precautions being observed, six samples were taken throughout the cheese, the whole approximating as near as possible 1 gram. The material was placed in a small weighed aluminum dish with a tight fitting cover. The dish was again weighed, and the weight of the cheese determined. The sample was then ground with sand in a sterile glass mortar, and with the aid of a glass rod fitted at one end with a rubber policeman, was washed into a large-mouthed bottle using a known quantity of sterile water. As before (8) an electrically-driven horizontal shaker was used for the mixing of the dilution. The mixing occupied five minutes, and to provide against organisms being carried down by sand, shaking by hand was done for another five minutes. The higher dilutions were then made in water blanks, and it was found that dilutions of 1:500,000 and 1:2,000,000 gave in practice the most satisfactory plates.

Comparative values of certain media

In the work on the "Kingston cheese" reported in 1922 (8) the indications were that glucose agar would give better results than lactose agar. At that time there was not sufficient data on which to form any definite conclusions. During the work herein recorded a comparison was made of glucose agar with lactose agar; and, later of glucose agar with the milk agar of Ayers and Mudge (1). Glucose agar proved to be the most satisfactory medium. Glucose gelatin plates were also made, and as a rule, higher counts were obtained than when using glucose agar. On hot days, however, it was very difficult to keep the gelatin from melting. As is stated on page 556, the dilution method was employed when using litmus milk and the Voges-Proskauer broth respectively. In many instances growth in higher dilutions was observed in the litmus milk than was the case with the Voges-Proskauer medium.

QUANTITATIVE BACTERIOLOGICAL ANALYSES

All quantitative examinations were made in triplicate and the results given below and in table 1 are averages of these three counts. The gelatin plates were incubated for five days at room temperature. The bile-salt broth tubes and bile-salt-agar plates were incubated for forty-eight hours at 37.5°C. All other media were incubated for five days at 22°C. In order to form some idea of the percentage of acid-formers present on the plates, litmus and brom-cresol-purple were used as

TABLE 1

DATES ON WHICH CHEESE WERE MADE	COUNTS MADE ON GLUCOSE AGAR NUMBER OF BACTERIA PER GRAM OF CHEESE	
	Green cheese*	Ripe cheese†
December 12, 1921	565,984,000	353,270,000
August 3, 1923	2,175,000,000	18,000,000
August 7, 1923	2,803,000,000	100,000,000
August 10, 1923	131,100,000	62,150,000
October 3, 1923	110,970,000	55,561,000
October 10, 1923	91,274,000	45,396,000
October 11, 1923	12,824,000	61,000,000
October 18, 1923	123,200,000	68,665,000
October 22, 1923	74,851,000	20,521,000
October 29, 1923	154,452,000	29,016,000

* "Green cheese" refers to the cheese not older than two days from time of making.

† "Ripe cheese" refers to the cheese ready for market, eight to twelve days from time of making.

indicators in a number of glucose-agar-plates. As was experienced before (8) in many instances the indicator in the whole of the medium was changed. On plates where the acid-formers could be differentiated, the number in the majority of cases was 100 per cent of the count and the proportion was not below 97 per cent in any examination.

Twenty cheeses were submitted to a quantitative bacterial analysis. On ten different days the bacterial content of a specific cheese of a certain day's "make," was determined, when the cheese was about twenty-four hours old; and another cheese

of the same day's "make" respectively was examined when ripe about ten days later. It was not possible to examine the same cheese when twenty-four hours old and then ten days old, because the size of the cheese, and the method of sampling, defined on page 557 necessitated the cutting up of the entire cheese for the first determinations. It is to be observed, however, that all the cheese made on any one day were from one vat of milk and from one body of curd which remained in the vat up to the filling of the moulds prior to pressing. Harrison and Connell in their work on cheddar cheese (5) state that

> A source of error in the quantitative bacteriological analysis of cheese is the fact, ... that plugs from different parts of the same cheese, of the same age, vary as much as 30 per cent, in the bacterial content. Further, even in the same plug, portions of equal weight sometimes show as high as 20 per cent of difference in the number of bacteria contained in it.

As yet no attempt has been made to determine if there were any great variations in numbers of bacteria between two cheeses of the same day's "make," but it is to be expected, considering that they were from the same curd, that no greater variation would be found than the 30 per cent found in different parts of a cheddar cheese by Harrison and Connell. In every case, excepting the cheese made on October the eleventh, a considerable reduction was shown in the bacterial count of the ripe cheese when compared with the count of the green cheese of the same day's "make." The number of organisms per gram varied considerably in the different cheeses examined. One cheese a day old gave a count as high as 4,000,000,000 microörganisms per gram; and another, also a day old, had a count as low as 9,000,000 microörganisms per gram. The counts in the ripe cheese varied from 147,000,000 to 12,000,000 per gram. Though there was this great difference in the number of organisms found in the different cheeses, there was no great difference in the quality of the cheese as judged for market. More detailed results will be found in table 1.

As in the examinations of two years ago there has been no attempt to make anaerobic examinations of the cheese. It is fully realized that this part of the work should be undertaken; but it was not possible simultaneously to do both aerobic and anaerobic examinations, and it was decided for the time being to concentrate on work under aerobic conditions. It is to be desired that an inquiry into the anaerobic flora of the cheese shall be instituted.

An effort was made to conduct histological examinations of the cheese in order to get some idea of the grouping of the organisms; and to secure counts of the bacteria by the microscopic method as recommended by Hucker (6). Thus far, time has not permitted the doing of other than preliminary work on this phase of the investigation. At a later date it is to be desired that the examination of histological specimens shall also receive consideration.

QUALITATIVE BACTERIOLOGICAL ANALYSES

Colonies which appeared to occur with the greatest frequency were taken off the plates and retained in pure culture. A large percentage of these colonies were similar to the small sub-surface and surface colonies—group II—reported in the studies undertaken in 1922 (8). Great difficulty was found at that time (8) in keeping the organisms of group II alive long enough to do even a preliminary examination of them. Also, the growth on agar slants was found to be insufficient for inoculation purposes. In order to avoid the difficulties mentioned above, the following procedure was evolved and followed with good results throughout the present investigation: The colonies were fished off gelatin plates in the manner found so advantageous in the later stages of the work reported before (8); a small lump of gelatin containing the colony was lifted out of the plate, put into Voges-Proskauer broth and held at 37.5°C. till the gelatin melted, freeing the colony. The tubes of broth were then incubated at 22°C. When a cloudiness in the broth indicated that growth had taken place, this broth was used to inoculate the desired culture media. A small quantity of the broth was drawn up into a sterile pipette

and all tubes of culture media inoculated with one drop each from the point of the pipette. The glucose broth of the Voges-Proskauer test was used because it was the only liquid medium in which the organisms of group II (8) could be grown satisfactorily.

As a preliminary examination, all cultures were stained by Gram and the reactions to litmus-milk, glucose, lactose and sucrose were recorded. Later it seemed desirable to determine the reaction to maltose, glycerol, salicin and mannitol. The results of the above examinations are recorded in table 2. The agar slants recommended by Conn and Hucker (3) and given in the "Manual of Methods for Pure Culture Study of Bacteria" (14) were used for the determination of acid and gas in all the carbohydrate media employed.

It has been noted above, that, for these determinations, one drop, from a finely-bored pipette, of the Voges-Proskauer glucose solution was used for the inoculation in each case. The possibility of the infinitesimal amount of glucose present in the drop of culture being sufficient to show acidity in the media other than glucose suggested itself. To check this, ten tubes of nutrient agar slants containing indicator, but no sugar, were inoculated at the same time as the other media. The control tubes remained neutral. Ten tubes of nutrient-agar were not considered as sufficient check, but the fact that a large percentage of the cultures did not produce acid on lactose-agar was considered as conclusive proof that when the minute quantity of glucose present in the drop of inoculating material was fermented there was not sufficient acid produced to change the color of the indicator in the media inoculated.

One hundred seventeen organisms were isolated from plates made of the cheese, and 5 organisms from plates made of the starter used in the making of the cheese. When the reactions of the cultures to maltose, salicin, glycerol and mannitol were determined, it was found that 23 organisms had died. The information already obtained about them, however, was enough to show to which main group they belonged. The 117 organisms divide themselves into five main groups.

TABLE 2

Morphology, cultural features and physiological reactions of organisms isolated from "Kingston cheese"

CULTURE NUMBER	NUMBER OF CULTURES REPRESENTED	MORPHOLOGY	GRAM STAIN	MOTILITY	CHROMOGENESIS WINSLOW'S CHART	GELATIN LIQUEFIED	GLUCOSE	LACTOSE	SUCROSE	GLYCEROL	SALICIN	MANNITOL	MALTOSE	LEVULOSE	RAFFINOSE	GELACTOSE	ARABINOSE	TREHALOSE	DEXTRIN	INULIN	DULCITOL	INDOL	METHYL RED	VOGES-PROSKAUER	NITRATES REDUCED	MILK			
																										Acid	Gas	Clot	Peptonized
115	6	Cocci 0.75-1 μ in diameter	+			−	+ −	+ −	− −																	+	−	+	−
121	16	Cocci 1-1.5×0.75-1 μ	+			−	+ −	+ −	− −																	+	−	+	−
212	1	Cocci 1-1.5×0.75-1 μ	+			−	+ −	+ −	− −	− −	− −	− −	− −													+	−	+	−
136	14	Cocci 0.75-1 μ in diameter	+			−	+ −	+ −	− −	− −	− −	− −	+ −													+	−	+	−
135	32	Cocci 1-1.5×0.75-1 μ	+			−	+ −	+ −	− −	− −	− −	− −	+ −													+	−	+	−
172	3	Cocci 1-1.5×0.75-1 μ	+			−	+ −	+ −	− −	− −	+ −	− −	+ −													+	−	+	−
156	5	Cocci 0.75-1 μ in diameter	+			−	+ −	+ −	+ −	− −	+ −	+ −	+ −													+	−	+	−
152	2	Cocci 1-1.5×0.75-1 μ	+			−	+ −	+ −	+ −	− −	+ −	+ −	+ −													+	−	+	−
101	3	Rods 1 μ×1-10 μ	+			−	+ −	+ −	+ −	+ −	+ −	+ −	− −		+ −											+	−	+	−
102	1	Rods 1 μ×1-10 μ	+			−	+ −	+ −	+ −	− −	+ −	+ −	+ −	+ −	+ −	+ −	+ −	+ −	+ −	+ −						+	−	+	−
103	2	Rods 1 μ×1-10 μ	+			−	+ −	+ −	+ −	+ −	+ −	− −	− −		+ −											+	−	+	−
104	14	Rods 1 μ×1-10 μ	+			−	+ −	+ −	+ −	+ −	+ −	+ −	+ −		+ −		+ −	+ −	+ −							+	−	+	−
M12	2	Rods 1 μ×1-10 μ	+			−	+ −	+ −	+ −	− −	+ −	+ −	+ −		+ −		+ −	+ −	− −							+	−	+	−
M9	1	Rods 1 μ×1-10 μ	+			−	+ −	+ −	+ −	− −	+ −	+ −	− −		+ −											+	−	+	−
119	5	Short rods	−	−		−	+ +	+ +	+ −	+ −	+ −	+ −	+ −								+ −	−	−	−	+	+	−	+	−
124	3	Short rods	−	−		−	+ +	+ +	− −	+ +	+ +	+ +	+ +								− −	+	+	−	+	+	−	+	−
143	5	Short rods	−	+		−	+ +	+ +	− −	+ +	+ +	+ +	+ +								+ +	+	+	−	+	+	+	+	−
206	3	Short rods	−	−		−	+ +	+ +	+ +	+ +	+ +	+ +	+ +								+ +	−	−	−	+	+	+	+	−
110	1	Spore forming rods	−	+		−	+ −	+ −	+ −	+ −	+ −	+ −	+ −													+	−	−	−
133	1	Micrococci	+		Yellow	+	+ −	− −	− −	+ −	+ −	+ −	+ −													−	−	−	+
214	1	Staphylococci	+		White	+	+ −	− −	+ −																	−	−	+	−
148	1	Micrococci	+		White	−	+ −	+ −	+ −	− −	− −	+ −	+ −												+	+	−	+	−

Reactions: + positive; −negative; ++ acid and gas; +−acid; −− no action.

GROUP I

In this group are found organisms isolated from pin-head colonies growing more particularly under the surface of the media, Gram-positive, spherical, occurring in chains, ones and twos and in clumps. Each strain ferments glucose and lactose to acid, produces acid and clot in litmus milk and fails to liquefy gelatin. Seventy-nine strains find themselves in this group. Within the group, however, specific strains vary in the size of the cells and in the action on certain of the carbohydrates other than glucose and lactose. In table 2 the variations in size of cell and in the action on the carbohydrates other than glucose and lactose are recognized and sub-groups are established. Of the 79 strains thus placed in sub-groups, cultures 115, 121, 212, 136, 135, 172, 156 and 152 prove to be representative of the number of strains respectively, as shown in the second column of the plate. Cultures 156 and 152 representing 5 and 2 strains respectively, agree in the essentia' characteristics with the description of Streptococcus lactis (Lister) according to Bergey's Manual of Determinative Bacteriology (13). Strains represented by Cultures 115, 121, 212, 136, 135 and 172, however, cannot be classified as typical forms of Streptococcus lactis (Lister) because of the failure to act on salicin, on mannitol, or on both (13). It was also noted that only 3 strains coming in this group and isolated from cheese, ferment salicin and mannitol to acid, while three more strains ferment salicin only. It may be that due to having passed through cheese the majority of the organisms which find themselves in group I had lost the power of fermenting salicin and mannitol, 3 strains had lost the power of fermenting mannitol only, while 3 strains had retained the power of fermenting both these carbohydrates. It would seem that with forms of Streptococcus lactis (Lister) attenuation takes place in passing through cheese. Support for this suggestion is found in the work of Lloyd on cheddar cheese (9). From well-matured cheese he isolated strains of true lactic acid producing organisms which he defined as Bacillus acidi lactici—now considered as Streptococcus lactis (Lister) (13) (9)—which failed to clot milk. Further, of the

organisms isolated from cheese 3 only of the strains included in this group ferment salicin and mannitol to acid, while three of the strains ferment salicin to acid but fail to act on mannitol. The remainder of those cultures isolated from cheese and included in this group have no action on either salicin or mannitol. Of the organisms comprising group I, every strain is found to be identical with one or more of the strains isolated by Hucker from cheddar cheese (7). Each of his strains is classified as Streptococcus lactis (Lister) (7). In studying the organisms placed here in group I, the reactions to salicin, mannitol and maltose, have been determined in addition to the reactions recorded by Hucker for his strains. Consequently, while Hucker (7) classified his organisms as Streptococcus lactis (Lister) the additional reactions recorded for the cultures—group I—of this report permit of a more specific classification, following Bergey (13). Accordingly, cultures 152 and 156 are placed as typical forms of Streptococcus lactis (Lister) (13), after Bergey; and Cultures 115, 121, 212, 136, 135 and 172, on account of their failure to ferment mannitol or salicin, or mannitol and salicinin, are placed as attenuated forms of Streptococcus lactis (Lister) (13).

GROUP II (GRAM-POSITIVE ROD-SHAPED ACID FORMERS)

Nineteen cultures represented in table 2 by cultures 101, 102, 103, 104, M12 and M9 are Gram-positive non-spore bearing rods with round ends, varying in length from 1 to 10μ, the majority approximating the minimum length rather than the maximum. There was a tendency to form short chains in the broth as used for the Voges-Proskauer test and to a somewhat lesser extent on agar. Good growth took place both at 22° and 40°C. All the cultures, with the exception of cultures 204 and 205 were picked off agar and gelatin plates incubated at 22°C. for five days, while cultures 204 and 205 were taken off plates incubated at 40°C. The strains do not liquefy gelatin but form a clean acid clot in litmus-milk in from fourteen to eighteen days at 40°C. followed by bleaching. Gas is not formed in any of the carbohydrates and glucose, lactose, sucrose, salicin and raffinose are fermented to acid. These cultures of group II seem

to bear a marked resemblance to Orla-Jensen's Streptobacterium (10) in that they are Gram-positive rods tending to form chains, growing at 22° to 40°C. and fermenting salicin to acid. Smears made from nutrient agar slants showed a large number of short rods which might be mistaken almost for diplococci with a number of rods about 10μ long. These smears were almost identical in appearance with Orla-Jensen's Streptobacterium plantarium number 18, agar streak one day at 30° plate XLII (10). According to Bergey's Manual (13) the cultural and morphological characteristics of the organisms here under discussion would place them in the genus Lactobacillus (Beijerinck) (13). It was impossible to isolate organisms of this type from the freshly made cheese though several attempts were made. All the cultures mentioned above were from cheese eight to ten days old. It has been known that Lactobacilli are found in mature cheddar cheese and it is interesting to know, that in a cheese which ripens so quickly, Lactobacilli are present in quite considerable numbers.

Cultures 101 and M9 do not ferment maltose, but ferment mannitol to acid. The cultural and morphological characteristics of these two organisms would seem to warrant the classifying of them as of the Lactobacillus bulgaricus (Grigoroff) type (13) and as of Rahe's type "D" (13).

Cultures 102 and 104 ferment maltose, mannitol, raffinose, dextrin, arabinose and trehalose to acid, trehalose being fermented slowly. The cultural characteristics of these organisms, and particularly their action on the carbohydrates, would require that they be classified as Lactobacillus cucumeris (Henneberg) (13). However, the length of the cells of cultures 102 and 104 vary from 1 to 10μ while the length of the cells recorded for Lactobacillus cucumeris are from 1.5 to 2μ. With the exception of the reaction to trehalose, the morphological and cultural characteristics of cultures 102 and 104 appear to be identical with those recorded for Lactobacillus plantari (Orla-Jensen) (13). Further these two organisms and Lactobacillus plantari, clot milk, while Lactobacillus cucumeris does not. The action of Lactobacillus cucumeris on dextrin is much slower as compared with its reaction to trehalose, while with strains 102

and 104, the reverse is the case. Trehalose is the sugar which, according to Bergey (13) divides Lactobacillus cucumeris and Lactobacillus plantari. It is obvious, therefore, considering the characteristics of Lactobacillus cucumeris and Lactobacillus plantari respectively, that cultures 102 and 104 are to be placed with one or other of these two known strains according to the relative importance which is to be attached to the fermentation of trehalose on the one hand, and to the size of cells and the clotting of milk on the other hand. On the whole following Bergey, the evidence submitted appears to be in favour of Lactobacillus cucumeris; and cultures 102 and 104 are classified as types of Lactobacillus cucumeris (Henneberg) after Bergey (13).

Culture 103 does not ferment maltose and mannitol. The action on the carbohydrates would place culture 103 as either Lactobacillus caucasicus (Kern) (13) or Lactobacillus boas-oppleri (Boas and Oppler) (13). As neither Lactobacillus caucasicus (13) nor Lactobacillus boas-oppleri will grow on gelatin at 22°C. and as culture 103 was isolated from a gelatin plate incubated at room temperature, it cannot be classified either as Lactobacillus caucasicus or Lactobacillus boas-oppleri, but is placed within the type species Lactobacillus caucasicus (Kern) (13).

Culture M12 ferments maltose, mannitol and raffinose to acid. The ability of this organism to ferment raffinose without fermenting dextrin does not permit of it being classified by Bergey's Determinative Bacteriology (13) other than as being a member of the genus Lactobacillus and coming within the type species Lactobacillus caucasicus (Kern) (13).

GROUP III (GRAM-NEGATIVE NON-SPORE FORMING RODS, WHICH FERMENT LACTOSE TO ACID AND GAS)

There are 16 organisms in this group represented on table 2 by cultures 119, 124, 127 and 206. All were short Gram-negative rods which ferment glucose and lactose to acid and gas, clot milk, reduce nitrates to nitrites, but fail to liquefy gelatin. The characteristics noted above, and the negative reaction to the Voges-Proskauer test would place these strains in the genus Escherichia (13).

Of the organisms in this group, culture 119 is a non-motile rod which ferments sucrose, salicin, maltose, glycerol, dulcitol and mannitol to acid; produces sliminess on agar, in peptone broth and in milk, but fails to produce either indol or acetyl-methyl-carbinol. When first isolated this strain fermented sucrose to acid and gas, but after being kept on artificial media for some months lost the faculty of producing gas on this carbohydrate. In its ability to produce sliminess on certain media, to ferment glucose and lactose to acid and gas and its inability to produce indol, culture 119 shows a resemblance to Bacterium aerogenes (Escherich) as described by Buchanan and Hammer (2). Their Bacterium aerogenes is shown as fermenting carbohydrates, other than glucose and lactose, to acid and gas, while this strain ferments them to acid only. Bacterium aerogenes (Escherich) becomes Aerobacter aerogenes (Escherich) in Bergey's Determinative Bacteriology (13). Following Bergey (13) culture 119 cannot be classified as Aerobacter aerogenes, for it fails to produce acetyl-methyl-carbinol, but based on the sum of the characteristics recorded the strain finds itself within the genus Escherichia. Of the species in this genus included in Determinative Bacteriology (13) it appears that Escherichia astheniae presents the features with which the culture under discussion most closely aligns itself. This culture, therefore, is here considered as being an atypical form of Escherichia astheniae (Dawson) (13).

Culture 124 is a non-motile rod, ferments glucose and lactose to acid and gas, but fails to act on sucrose and salicin. But for the fact that culture 124 is non-motile, it could be classified as Escherichia paragrunthali (Castellani and Chalmers) (13). This lack of motility, however, prohibits it being classified as a true form of Escherichia paragrunthali and it is classified as of the type Escherichia paragrunthali (Castellani and Chalmers) (13).

Culture 143 is a strain of motile rods which forms indol, ferments salicin and dulcitol to acid and gas, but fails to ferment sucrose. The ability of this strain to reduce nitrates to nitrites, to ferment glucose, lactose, salicin and dulcitol to acid and gas, the inability to produce acid in sucrose and to form acetyl-

methyl-carbinol places this organism as a true type of Escherichia coli (Escherich) Castellani and Chalmers (13).

Culture 206 is a non-motile rod which ferments salicin and sucrose to acid and gas, but does not produce indol or give the Voges-Proskauer reaction. The ability of the culture to ferment sucrose and lactose to acid and gas, the inability to produce indol or give the Voges-Proskauer reaction, and its failure to show motility would suggest that it be placed as Escherichia astheniae (Dawson) (13). Escherichia astheniae, however, has no action on salicin, while culture 206, as noted above, ferments this carbohydrate to acid and gas. This difference in action on salicin would not permit the classifying of this strain as a typical form of Escherichia astheniae and it is classified here as an atypical form of Eschrichia astheniae (Dawson) (13).

GROUP IV (SPORE-BEARING RODS)

Only one spore-bearing organism, culture 110, was found in this investigation. The strain is a Gram-negative motile rod 2 to 4μ in length, the cells occurring singly and in pairs. The spores are terminal and the rods are swollen at sporulation giving the cell the appearance of a tadpole. The growth on agar is pale yellowish white after forty-eight hours, gelatin is not liquefied and acid is formed in milk but no clot is produced. Glucose, lactose, and sucrose are fermented to acid. The cultural and morphological characteristics of culture 110 appear to be identical with those recorded for Bacillus pseudotetanicus (Kruse) (13) with the exception of the action on the carbohydrates; Bacillus pseudotetanicus showing no action on the carbohydrates. Culture 110 bears a marked resemblance also to Bacillus circulans (Jordan) (13) but the 2 cultures do not agree in the reaction to the Gram stain. It is suggested that in spite of the difference noted with respect to the action to the Gram stain, culture 110 shall be considered as being of the type Bacillus circulans (Jordan) (13).

GROUP V (COCCUS FORMS)

Culture 133 is a minute Gram-positive coccus about 0.5μ in diameter, which liquefies gelatin, ferments glucose, maltose,

salicin and mannitol to acid, but has no action on lactose or sucrose. This strain bears a marked resemblance to Micrococcus lactis varians of Conn, Esten and Stocking (4). These investigators found that some forms of Micrococcus lactis varians did not ferment lactose and sucrose, characteristics applying equally to culture 133. Comparing the characteristics as a whole, the strain agrees even more closely with Micrococcus lactis varians (type A) of Conn, Esten and Stocking (4) both as to the formation of acid in glucose, and in the curdling and digesting of milk without the formation of acid. The resemblance, however, of the strain to Micrococcus varians (Dyar) Conn (13) is not so pronounced; yet Micrococcus varians (Dyar) Conn is considered in Bergey's Determinative Bacteriology as being synonymous with Micrococcus lactis varians of Conn, Esten and Stocking. Hence, it would seem that culture 133 is to be classified as of the type Micrococcus varians (Dyar) Conn (13).

Culture 148 is a strain of Gram-positive cocci growing in clumps. It fails to liquefy gelatin, forms a clean acid clot in milk, reduces nitrates to nitrites and ferments glucose, lactose and sucrose to acid. No action on maltose, salicin or inulin has been noted. The growth is good on artificial media and the pigment on agar after fourteen days, according to the Winslows' chart (16), is Light Cadmium Yellow, Chrom III, designated by these workers as "white." The characteristics recorded would suggest that the strain be placed in the genus Staphylococcus (13). To take the classification to a more specific stage, the failure of the organism to liquefy gelatin and the ability with which lactose is fermented indicate a close resemblance of the strain to Staphylococcus tetragenus (Koch-Gaffky) (13). The identity of the 2 strains cannot be accepted without qualification for culture 148 reduces nitrates to nitrites, and under the microscope appears as cells in clumps, while Staphylococcus tetragenus (13) does not reduce nitrates and the cells appear in groups of four. Winslow and Winslow (16) state that Albococcus tetragenus (Gaffky)—albococcus being a genus since absorbed in the genus Staphylococcus—is closely related to Albococcus candidus

(Cohn) and in describing Albococcus candidus, Winslow et al. (17) make the following statement

> The second type in abundance in our study, and the type found most commonly on the skin after St. epidermidis by Gordon, is the form which ferments lactose but fails to liquefy gelatin, identified by Winslows as Albococcus candidus and now to be called Staphylococcus candidus. Our strain, however, reduced nitrates and generally clotted milk which Gordon's type did not. Three strains sent to the museum collection as Micrococcus tetragenus all belonged to this group. None of them reduced nitrates and results are variable in milk and in regard to ammonia production.

The strain recorded here agrees with the above description of Staphylococcus candidus with respect to the clotting of milk and the reduction of nitrates to nitrites. In table 10 of the same report (17) Winslow et al. show 20 of their strains as fermenting maltose. Culture 148 fails to ferment this sugar but in all other respects it appears to be identical with their cultures as cited. The organism originally described by Cohn was placed by him in the genus Micrococcus as Micrococcus candidus (16). Later organisms showing similar characteristics to Cohn's Micrococcus were placed by the Winslows (16) within the type center Albococcus candidus (Cohn) and later as Staphylococcus candidus (Cohn) (17). The Committee of the Society of American Bacteriologists in Bergey's Determinative Bacteriology (13) have again placed organisms of this type in the genus Micrococcus, as Micrococcus candidus (Cohn) (13). Though culture 148 differs from Micrococcus candidus (Cohn) (13) in the action to nitrates, in the main the characteristics are identical and culture 148 is classified here as of the type Micrococcus candidus (Cohn) (13).

Culture 214 is a Gram-positive coccus, occurring in irregular groups. The strain liquefies gelatin, stratiform, produces a white pigment on agar, ferments glucose and sucrose to acid, but no action on lactose can be noted. This culture died before it could be investigated further. According to the cultural and morphological features determined, however, culture 214 would be placed in the genus Staphylococcus, and of the type species

Staphylococcus aureus (Rosenbach) (13). The inability of the strain to ferment lactose, does not permit of it being classified more specifically. Winslow, Rothberg and Parsons in the White and Orange Staphylococci (17) show 10 cultures in their table 8, which are white pigment formers, liquefying gelatin, but failing to ferment lactose. Later the same workers say (17): "The lactose-negative gelatin-positive type of white pigment producers appears in our study, as in that of Gordon, to be a rarer one, and this form, as well as the forms which exhibit miscellaneous fermentative reactions may best be left for the present without specific names." It is felt that culture 214 should be included in this group. Culture 214 is, therefore, left for the present as of the type species Staphylococcus aureus (Rosenbach) (13).

OBSERVATIONS

The technique evolved when engaged on the work in 1922 (8) has been adopted throughout the present investigation with pronounced satisfaction. Further, the present paper confirms the previous findings (8) that for the determinations of the bacterial flora of "Kingston cheese" glucose-agar and glucose-gelatin are the most satisfactory media.

The total number of organisms present in the "Kingston cheese" both in the cheese when newly made and the cheese when ten days old, is found to approximate very closely the numbers recorded in the work on cheddar cheese. Though the total number of organisms present in the "Kingston cheese" ten days old is not constant, and may vary, as this investigation shows from 20,000,000 to 350,000,000 bacteria per gram, the cheese examined in each case were normal both as to texture and flavor.

Though no definite effort was made to determine the percentage of acid formers, the results obtained showed that at least 97 per cent of the flora were acid formers.

This paper would seem to indicate that the organisms which occur with the greatest frequency in the cheese when one day old are those of the Streptococcus lactis (Lister) type, and in cheese of the same day's "make" when ten days old are those

of the Streptococcus lactis (Lister) type and those of the Lactobacillus (Beijerinck) type. It appears to be well established that the successful ripening of cheddar cheese is very largely dependent upon the bacterial flora determined according to the recorded literature. The work herein presented, quite definitely shows that the flora of the "Kingston cheese" is very similar, if not identical with the flora recorded for cheddar cheese. Yet in the case of the "Kingston cheese" we have a cheese which is mature in ten days after making, while a cheddar cheese requires from three to six months to arrive at maturity.

It would seem, therefore, that in contemplating the factors determining the successful ripening or maturing of the "Kingston cheese" due regard must be paid to the system of manufacture adopted, to the temperature at which ripening takes place and to all the processes associated with the management of the cheese, for as far as this paper can define, the bacterial flora of the "Kingston cheese" is clearly identical with that of the cheddar cheese.

SUMMARY

A brief résumé is given of the system adopted in the making of the "Kingston cheese" and the specific characteristics of the cheese are noted.

Bacteriological analyses have been made of twenty cheeses manufactured on ten different days, ten cheeses when one day old and ten cheeses of the same day's "make" when mature at ten days after making. The results of the analyses made of these twenty cheeses are recorded in table 1.

A list of the media employed is recorded and a description of the method of examination of the cheese is given. Several varieties of media were used with the object of determining which were best adapted for the determination of the bacterial analyses of the "Kingston cheese," glucose-agar and glucose-gelatin proving to be most satisfactory.

One hundred seventeen organisms have been isolated from plates made of the cheese and 5 from plates made of the starter used in the making of the cheese. As far as possible the organ-

isms were those which appeared to occur with the greatest degree of frequency. Though 18 of the 122 organisms isolated were of the genus Escherichia this does not necessarily represent the percentage of organisms of that Genus present in the cheese. The strains were taken from MacConkey's broth tubes and MacConkey's agar plates in order to determine the species of gas formers occurring with the greatest frequency.

The morphology, cultural features and physiological reactions of organisms isolated from "Kingston cheese" are given in table 2.

The 122 organisms isolated are placed in five main groups, and are classified according to Bergey's Determinative Bacteriology (13).

Group I—Streptococcus lactis (Lister) types

Seventy-nine of the 117 cultures recorded in this paper find themselves in this group, and are classified as follows.

Cultures 156 and 152 representing in all 7 strains are classified as Streptococcus lactis (Lister) (13).

Cultures 115, 121, 212, 136, 135, and 172, representing 72 strains are placed as attenuated forms of Streptococcus lactis (Lister) (13).

Group II—Lactobacillus (Beijerinck) types (13)

Twenty-three strains are placed in this main group and are classified as to species.

Cultures 101 and M9 representing 4 cultures are classified as Lactobacillus bulgaricus (Grigoroff) (13) and of the type "D" (Rahe) (13).

Cultures 102 and 104 representing 15 strains are placed as atypical forms of Lactobacillus cucumeris (Henneberg) (13).

Cultures 103 and M12 representing 2 strains each are classified as of the type species Lactobacillus caucasicus (Kern) (13).

Group III—Gram-negative, lactose, fermenting rods

In all there are 16 strains in this group represented by 4 cultures.

Eight strains represented by cultures 119 and 206 are classified as atypical forms of Escherichia astheniae (Dawson) (13).

Three strains represented by culture 124 are considered as atypical forms of Escherichia paragrunthali (Castellani and Chalmers) (13).

Five strains represented by culture 143 are classified as Escherichia coli (Escherich) Castellani and Chalmers (13).

Group IV—Spore-bearing rods

Only 1 culture of spore-forming rods was found in this investigation.

Culture 110 is placed as an atypical form of Bacillus circulans (Jordan) (13).

Group V—Coccus forms other than Streptococcus lactis (Lister)

Three cultures find themselves in this group, 2 of which are Micrococci and 1 a Staphylococcus.

One strain, culture 133, is classified as of the type Micrococcus varians (Dyar) Conn (13).

One strain, culture 214, is placed as of the type species Staphylococcus aureus (Rosenbach) (13).

One strain culture 148, is considered to be within the type species Micrococcus candidus (Cohn) (13).

Observations on the data presented are offered.

ACKNOWLEDGMENTS

I wish to thank Prof. Wilfrid Sadler, at whose suggestion this work was commenced, with whom I have consulted from time to time, and who has kindly read over the manuscript.

This investigation has been made possible by laboratory facilities, which have been placed at my disposal by the University of British Columbia.

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A COMPARATIVE STUDY OF METHODS FOR DETERMINING TOTAL SOLIDS IN ICE CREAM

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Received for publication September 10, 1924

In this paper are presented a second series of results involving the comparative study of methods for determining total solids in dairy products. The first report on this study was made by Fisher and Rice (1) and dealt with the determination of total solids in sweetened condensed milk. As reported, twenty samples of condensed milk were tested for total solids by the official, the Mojonnier and a modified method suggested by the authors. Considering the properties of sweetened condensed milk, the three methods gave reasonably close results. The Mojonnier method averaged 0.57 per cent and the modified 0.47 per cent higher than the official. In view of these results and the greatly felt need for a simple, economical, but accurate total solids test for ice cream, the second series of trials were made on this dairy product.

EXPERIMENTAL WORK

Fifty samples of ice cream were analyzed by both the Mojonnier and a modified test similar to the one suggested for sweetened condensed milk (1). Since there is no recognized official test available, the authors adapted a total solids test from the official method for sweetened condensed milk. In the following pages this method is referred to as "adapted official." Twelve of the samples of ice cream were tested for total solids by the adapted official and compared with the Mojonnier and the modified method.

The procedure of each was as follows.

1. Adapted test from official method for condensed milk

One-half pint samples of ice cream were gradually warmed by placing in a hot water bath, the temperature of which was not over 30°C. Samples were allowed to remain in the water bath until they reached about 25°C. and were thoroughly melted. Exactly 20 grams of thoroughly mixed sample were weighed into a beaker, and transferred to a 100 cc. volumetric flask with the aid of hot redistilled water. This was cooled and made up to the mark with redistilled water. Exactly 10 cc. of the solution was pipetted into a previously dried and tared aluminum dish and the free moisture evaporated off over water bath. This required about twenty minutes. Dishes were then transferred to a water oven and drying continued at the temperature of boiling water until constant weight was reached.

The dishes used in this test were ordinary flat-bottomed aluminum dishes about 5 cm. in diameter. Empty dishes were dried in the water oven and then cooled for a similar period in an ordinary calcium chloride desiccator; they were always kept covered while weighing. The percentage of solids were calculated from the final weight of solids in the dish.

2. Modified method (as adapted from modified method for sweetened condensed milk)

The sample was prepared in a similar manner as that outlined above by melting at about 25°C. in water bath. Approximately 1 gram of well mixed sample was weighed directly into a previously dried and tared aluminum dish. One cubic centimeter of hot redistilled water was added and thoroughly mixed. The procedure from this step on was similar to the modified test for sweetened condensed milk and was briefly as follows:

After adding the distilled water, the dishes were placed on an electric hot plate, kept at 180°C. and the moisture carefully evaporated off until first traces of brown appeared. Drying was then continued in a water oven at the temperature of boiling water until constant weight was reached.

The dishes used in this modified test, as in the adapted official test, were ordinary flat-bottomed aluminum dishes about 5 centimeters in diameter. Empty dishes were dried for ten minutes in water oven and then cooled for a similar period in an ordinary calcium chloride desiccator. They were always kept covered while weighing.

3. The Mojonnier method

The procedure for determining total solids in ice cream as described in Technical Control of Dairy Products by Mojonnier and Troy, page 126, was followed exactly.

EXPERIMENTAL RESULTS

The percentage of total solids obtained by each of the three methods for twelve samples of ice cream are reported in table 1. A study of the table shows that in most cases the results obtained by the three methods compare fairly closely. In all but the first two samples the Mojonnier method gave the highest results, the adapted official the lowest, while the modified method usually gave results between the adapted official and the Mojonnier. It is interesting to recall that the same observations were made in regard to the results obtained by the three methods in the case of sweetened condensed milk.

Table 2 shows the variation in the modified and Mojonnier methods from the adapted official. On 12 samples the Mojonnier test averaged 0.44 per cent higher than the adapted official while the modified test averaged 0.23 per cent higher. Fifty-eight per cent of the results by the modified test checked within 0.25 per cent with the adapted official while all checked within 0.5 per cent. In the case of the Mojonnier 16.6 per cent checked within 0.25 and 50 per cent checked within 0.5 per cent.

If one takes the adapted official as the standard for comparison, the modified test gave more favorable results than the Mojonnier. Furthermore as already pointed out in the first paper (1), the modified method is simpler and more rapid than the official. The equipment needed is inexpensive and frequently on hand in the laboratory.

TABLE 1

Percentage of solids in samples of ice cream according to three methods studied

SAMPLE NUMBER	ADAPTED TEST FROM OFFICIAL METHOD FOR SWEETENED CONDENSED MILK	MODIFIED METHOD	MOJONNIER
1a	36.71	36.51	36.72
1b	36.64	36.58	36.68
2a	38.01	38.27	38.51
2b	38.10	38.17	38.55
3a	38.05	38.21	38.31
3b	38.04	38.19	38.33
4a	40.39	40.59	40.86
4b	40.35	40.48	40.88
5a	40.07	40.41	40.62
5b	40.04	40.47	40.64
6a	38.03	38.37	38.80
6b	38.05	38.42	38.92

TABLE 2

Variation percentage of solids in ice cream as obtained by the three methods studied

SAMPLE NUMBER	MODIFIED FROM ADAPTED OFFICIAL	MOJONNIER FROM ADAPTED OFFICIAL
1a	−0.20	+0.01
1b	−0.06	+0.04
2a	+0.26	+0.54
2b	+0.07	+0.45
3a	+0.16	+0.26
3b	+0.15	+0.29
4a	+0.20	+0.47
4b	+0.13	+0.53
5a	+0.34	+0.55
5b	+0.43	+0.60
6a	+0.34	+0.77
6b	+0.37	+0.87
Average variation.....	0.226	0.483
	58 per cent within 0.25 per cent of adapted official 100 per cent within 0.50 per cent of adapted official method	16.6 per cent within 0.25 per cent of adapted official 50 per cent within 0.50 per cent of adapted official method

The modified test therefore would recommend itself as a simple, economic and accurate total solids test for use in ice cream factories and laboratories not provided with the equipment required for the Mojonnier method.

Realizing that results on twelve samples are not sufficient data on which to draw definite conclusions thirty-eight additional samples of ice cream were analyzed for total solids by the modified and Mojonnier method. We were desirous also of analyzing these samples by the adapted official, but this test while undoubtedly accurate and reliable is laborious and time consuming and therefore not well suited for factory work.

As the modified method compared closely with the official in both the 20 samples of condensed milk (1) and the 12 samples of ice cream it was decided to compare only the modified and the Mojonnier method in the next series of trials.

The percentages of total solids obtained by these two methods on 50 samples of ice cream are given in table 3. The results on these 50 tests confirm previous observations in that in all cases the modified method gave slightly lower results than the Mojonnier method. However, as brought out in the following summary, the two methods checked reasonably closely:

34 samples or 68 per cent checked within 0.25 per cent
40 samples or 80 per cent checked within 0.30 per cent
47 samples or 94 per cent checked within 0.40 per cent
48 samples or 96 per cent checked within 0.50 per cent

The average variation as pointed out at the bottom of table 3, was 0.223 per cent. This means that on 50 samples the modified test averaged 0.223 per cent lower than the Mojonnier method. When it is recalled that the modified averaged 0.226 per cent higher than the adapted official method, this fact becomes significant. It means that the modified method gives results about half way between the adapted official and the Mojonnier method.

In view of these facts, it seemed desirable to further standardize the procedure of the modified method, by establishing the average time required for the ice cream to reach constant weight.

For this purpose observations as to the time required to reach constant weight were made on 23 samples of ice cream.

The dishes containing the ice cream were first weighed at the end of two hours drying in the oven and then again at intervals

TABLE 3

Percentage of solids and variation percentage of solids as obtained by the modified and Mojonnier method for determining total solids in ice cream

SAMPLE NUMBER	MOJONNIER METHOD	MODIFIED METHOD	MODIFIED FROM MOJONNIER	SAMPLE NUMBER	MOJONNIER METHOD	MODIFIED METHOD	MODIFIED FROM MOJONNIER
1	36.74	36.54	−0.20	26	38.74	38.43	−0.31
2	38.53	38.22	−0.29	27	37.41	37.29	−0.12
3	38.32	38.20	−0.12	28	38.22	38.10	−0.12
4	40.87	40.59	−0.28	29	41.49	41.25	−0.24
5	40.82	40.61	−0.21	30	37.39	36.95	−0.44
6	40.83	40.50	−0.33	31	41.45	41.25	−0.20
7	40.85	40.43	−0.32	32	38.12	37.94	−0.18
8	40.85	40.67	−0.18	33	38.66	38.62	−0.04
9	40.86	40.66	−0.20	34	35.73	38.55	−0.18
10	40.84	40.44	−0.40	35	39.76	39.61	−0.15
11	38.87	38.63	−0.24	36	38.70	38.40	−0.30
12	38.85	38.64	−0.21	37	39.07	38.86	−0.21
13	38.35	38.23	−0.12	38	42.50	42.41	−0.09
14	38.54	38.42	−0.12	39	38.62	38.34	−0.28
15	38.37	38.20	−0.17	40	37.28	37.00	−0.28
16	39.97	39.59	−0.38	41	35.82	35.60	−0.22
17	39.00	38.24	−0.66	42	35.43	35.17	−0.26
18	36.90	36.77	−0.13	43	40.62	40.47	−0.15
19	36.89	36.69	−0.20	44	38.80	38.42	−0.38
20	39.37	39.15	−0.22	45	38.19	38.00	−0.19
21	37.99	37.65	−0.34	46	35.37	35.14	−0.23
22	38.07	37.86	−0.21	47	36.61	36.52	−0.09
23	36.74	36.63	−0.11	48	38.86	38.63	−0.03
24	35.67	35.11	−0.56	49	38.64	38.49	−0.15
25	37.12	37.00	−0.12	50	39.12	39.10	−0.02
Average variation.....							0.223

of thirty minutes until constant weight was reached. Results are tabulated in table 4. If one considers samples that check within 0.05 per cent as having reached constant weight, all had reached constant weight at the end of three hours drying. However, 15 samples or 65 per cent had reached constant weight

at the end of two hours drying in the water oven. Of the other 8 samples 7 lost less than 0.20 per cent from the time they had been dried for two hours until they reached constant weight.

Since for practical factory purposes, tests for total solids are usually satisfactory when they check within 0.25 of one per cent

TABLE 4

Time required for solids in ice cream to reach constant weight in modified test

SAMPLE NUMBER	PER CENT OF TOTAL SOLIDS AFTER DRYING FOR				PER CENT MOISTURE LOST IN VARIOUS PERIODS OF DRYING IN WATER OVEN		
	2 hours	2½ hours	3 hours	3½ hours	2 to 2½ hours	2½ to 3 hours	3 to 3½ hours
1	37.79	37.76	37.65	37.65	0.03	0.11	0
2	35.11	35.07	34.99	34.99	0.04	0.08	0
3	36.90	36.88	36.81	36.81	0.02	0.07	0
4	37.54	37.54	37.42	37.42	0	0.12	0
5	38.75	38.75	38.62	38.62	0	0.13	0
6	38.57	38.50	38.32	38.32	0.07	0.18	0
7	38.66	38.63	38.54	38.54	0.03	0.09	0
8	37.14	37.14	37.14	37.14	0	0	0
9	38.25	38.21	38.14	38.14	0.04	0.07	0
10	39.60	39.60	39.60	39.60	0	0	0
11	42.41	42.41	42.41	42.41	0	0	0
12	37.00	37.00	37.00	37.00	0	0	0
13	35.56	35.56	35.56	35.56	0	0	0
14	38.32	38.27	38.27	38.27	0.05	0	0
15	40.45	40.41	40.41	40.41	0.04	0	0
16	38.37	38.37	38.37	38.37	0	0	0
17	37.96	37.87	37.87	37.87	0.09	0	0
18	40.63	40.58	40.58	40.57	0.05	0	0.01
19	40.50	40.50	40.48	40.48	0	0.02	0
20	40.69	40.69	40.69	40.69	0	0	0
21	40.60	40.60	40.57	40.57	0	0.03	0
22	40.43	40.43	40.43	40.43	0	0	0
23	38.66	38.62	38.62	38.62	0.04	0	0
Average loss of moisture.....					0.021	0.041	0

and since in commercial work the time required to make the test is frequently more important than absolute accuracy, it seems justifiable to consider two hours as sufficient for such purposes. Whenever possible and in all cases of careful analytical work samples should be dried for three hours.

It has been suggested by other workers that, as there is at present no offiical test for ice cream, as recognized by the Association of Official Agricultural Chemists, one of these methods should be proposed for acceptance as the official total solids tests for ice cream. Certainly in view of the increasing importance of the ice cream industry, there is need for a practical, accurate and reliable total solids test which can be considered as the official method. If such procedure seems desirable, the question arises as to which method should be recommended. For the sake of uniformity of methods, the adapted official test as suggested in this paper would seem well suited to meet the requirements, as it is based on a procedure similar to the official test for other dairy products. However, as already pointed out, this test while accurate and reliable, is laborious and time consuming, requiring from six to eight hours for completion. The modified method, as outlined in this paper, overcomes these objections. Basing conclusions on data secured, the authors believe the modified method is also accurate and reliable. Its simplicity and relative rapidity are features which tend to make it more practicable and acceptable than the adapted official method.

SUMMARY AND CONCLUSIONS

1. A total of 50 samples of ice cream was analyzed for total solids. Twelve of these samples were tested by three methods: (*a*) the Mojonnier, (*b*) a modified test similar to the one suggested for sweetened condensed milk, (*c*) a method adapted from the official method for determining solids in sweetened condensed milk. The other 38 samples were analyzed by the first two mentioned methods.

2. The Mojonnier method invariably gave higher results than the method adapted from the official test for condensed milk; the average of 12 samples was 0.483 per cent above.

3. The modified method in more than 80 per cent of the cases also gave somewhat higher results than the adopted official, but checked closer than the Mojonnier; in 12 samples the average difference was 0.226 per cent.

4. In comparing results on 50 samples analyzed by both the Mojonnier and the modified method, the Mojonnier invariably gave higher results. The average of these 50 samples was 0.223 per cent above the modified method.

5. Observations as to time required to reach constant weight in modified method seems to indicate that for all practical purposes such as determination of solids in commerical factories, two hours of drying in water oven is sufficient. Whenever time permits and in all cases of careful analytical work, samples should be dried for three hours.

6. The data as presented seems to warrant the conclusion that the modified method is a simple, economical and accurate method for determining the per cent of total solids in ice cream.

7. As at present there is no official method for determining the per cent of total solids in ice cream the two methods suggested (1) the modified and (2) the method as adapted from the official method for sweetened condensed milk should receive consideration, thought and study as to the desirability of having one of them recognized as the official method.

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THE USE OF TRUE AND IMITATION VANILLA EXTRACTS IN ICE CREAM[1]

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Received for publication July 17, 1924

Every manufacturer of ice cream has, at some time or another, been confronted with the problem of determining the relative merits of artificial and true bean vanilla extracts. It has been claimed that the artificial extract will satisfy the same wants and desires as the geniune product. If the latter statement were to be accepted, arguments would not arise. However, the demand for the true bean extract is increasing rapidly enough to prove that it is still considered superior to the imitation, and the increased cost will not deter the buyer, if he seeks the best.

Because of the distinct taste which flavoring extracts impart to ice cream, it is important that they be of the best quality. The expense of the true extracts has been instrumental in encouraging the manufacture of many cheap imitations, as well as the production of extracts which have been diluted, or made from inferior beans, the flavor of which is often increased by the addition of vanillin and coumarin. The question naturally arises—is the manufacturer of ice cream getting what he is paying for when he buys a vanilla extract; also, how can he determine whether the extract is genuine or artificial.

The objects of this study were to determine the relative flavoring ability of true and artificial vanilla extracts in ice cream, and to determine the chemical and physical differences of the various extracts.

[1] This paper is a preliminary report, published as Contribution No. 15 of the Department of Agricultural Chemistry, The Pennsylvania State College.

TABLE 1

Testing experiments made on vanilla extracts

SAMPLE NUMBER	JUDGE 1			JUDGE 2			JUDGE 3			TYPE OF EXTRACTS DETERMINED BY ANALYSIS
	Kind	Strength	Quality	Kind	Strength	Quality	Kind	Strength	Quality	
10	True	Weak	Poor	Imitation	Fair	Fair	Imitation	Fair	Good	True
26	True	Fair	Good	True	Good	Excellent	True	Strong	Good	Imitation
27	Imitation	Strong	Fair	Imitation	Strong	Poor	Imitation	Strong	Good	True
28	Imitation	Fair	Good	True	Strong	Excellent	Imitation	Strong	Fair	Imitation
19	Imitation	Strong	Fair	Imitation	Strong	Poor	Imitation	Strong	Poor	Imitation
2	Imitation	Strong	Poor	Imitation	Strong	Very poor	True	Fair	Good	True
5	True	Fair	Good	Imitation	Fair	Poor	Imitation	Fair	Poor	True
23	True	Weak	Poor	True	Fair	Good	Imitation	Fair	Poor	True
24	True	Weak	Fair	True	Weak	Poor	True	Weak	Poor	Imitation
6	True	Weak	Fair	True	Strong	Good	True	Weak	Poor	True

EXPERIMENTAL

Twenty-five samples of extracts were obtained from various manufacturers. Ten samples were chosen at random—nothing being known about their composition. Chemical analyses were made on twenty-five samples, but as only ten of these were used in the experimental ice cream, results are given on only ten samples.

The first test was made by tasting the extracts to be examined —just as they were obtained from the manufacturer. Several men tasted the samples, which were in numbered bottles bearing no label, in an effort to classify the extracts as either true or

TABLE 2

Tasting experiments made on ice cream containing true and imitation vanilla extracts

SAMPLE NUMBER	TYPE OF EXTRACT BY ANALYSIS	NUMBER OF TIMES SELECTED BY JUDGES				
		First	Second	Third	Fourth	Fifth
10	True	3	2	3	2	0
26	Imitation	6	3	1	0	0
27	True	3	6	1	0	0
28	Imitation	1	2	6	1	0
19	Imitation	0	0	0	0	10
2	True	3	4	3	0	0
5	True	7	3	0	0	0
23	True	2	3	2	3	0
24	Imitation	2	2	5	1	0
6	True	2	2	6	0	0

artificial, and to judge the quality. After this initial test an endeavor was made to select the best "true" flavor and the best "imitation." The results are listed in table 1.

Ice cream mixes were prepared in which the vanilla extract was the only variable. After freezing each mix, five samples from each freezing were removed from the freezer and placed in the hardening room. An initial tasting test was made at the time of freezing, and a sample was removed from the hardening room every week for five weeks to determine the effect of storage on the persistence of the flavoring quality. The results of these tests are shown in table 2.

Chemical and physical examinations were made of the various extracts. The specific gravity, total solids, ash, percentage of alcohol and lead number were determined quantitatively. Qualitative tests were also made to determine the presence of coumarin and the methods ordinarily used to detect artificial extracts (1) were employed. The methods outlined by the Association of Official Agricultural Chemists (2) were used to obtain the specific gravity, total solids and ash, while the Wichmann method (3)

TABLE 3

Results of analysis on vanilla extracts

SAMPLE NUMBER	TYPE OF EXTRACT BY ANALYSIS	SPECIFIC GRAVITY 20°/4°	TOTAL SOLIDS	ASH	PER CENT ALCOHOL	LEAD NUMBER	COUMARIN
10	True	1.016	5.91	0.05	30.0	0.584	
26	Imitation	1.006	14.13	0.063	26.4	0.362	Present
27	True	1.060	34.39	0.24	24.0	0.785	
28	Imitation	1.066	30.60	0.04	26.0	0.226	Present
19	Imitation	1.034	23.84	0.001	26.4	0.284	Present
2	True	1.065	31.37	0.21	33.6	0.894	
5	True	0.999	27.51	0.07	42.0	0.996	
23	True	1.003	24.08	0.001	38.4	0.56	
24	Imitation	1.049	23.20	0.05	34.2	0.412	
6	True	1.140	57.50	0.20	0	0.916	

was used to obtain the lead number and the percentage of alcohol. Coumarin was also tested for by means of the Wichmann method (4).

Table 3 shows the analytical data obtained on the examination of the ten extracts used in ice cream.

DISCUSSION OF RESULTS

The initial test made by tasting the various extracts failed to enable the judges to classify an extract as genuine or artificial (see table 1). It should be remembered that these tests were not made by men who were vanilla experts. All of them, however, have had experience in the manufacture of ice cream, and should compare very favorably in ability with the average man in the plant who has to buy or select the flavoring extracts. An ex-

pert vanilla taster could undoubtedly tell the the difference between the various grades of vanilla by tasting, but the average man cannot.

When the samples of ice cream were judged for flavor, those containing the true flavors were selected the most frequently as being best. The samples to be judged were labelled with numbers, so that the judges did not know the nature of the extract used. One imitation, no. 26 (see table 2), compared very favorably with the true extracts. The flavor of the imitations did not persist as well as that of the true extracts during storage. It will be noticed that there was a considerable variation in the selection of the true extracts, no. 5 standing at the head of the list. Here, again, much difficulty would be experienced if we were to try to classify the extracts as true or imitation.

On examining the data obtained by analysis (see table 3), it will be observed that results obtained overlap each other—that is, the data on the imitation extracts is similar to that of the true extracts—with the exception of the lead numbers. This fact should be borne in mind when analyzing samples of vanilla. The lowest lead number of a true extract was found to be 0.56 and the highest 0.996. The lowest lead number for an imitation extract was found to be 0.226 and the highest 0.412. The percentage of alcohol in genuine extracts varied from 0 to 42 per cent, while in the artificial preparations the range was between 26 and 34 per cent. No analyzed samples fulfilled the requirements of the United States Pharmacopoeia. Sample No. 5, which had the highest lead number, also received highest rank in the tasting tests made on the ice cream samples.

SUMMARY

1. Tasting extracts will not enable the average ice cream manufacturer to determine the difference between a true and an artificial extract.

2. Tasting samples of ice cream will not enable the average ice cream manufacturer to tell whether or not a true or an artificial extract has been used in ice cream.

3. The experiments tend to show that true flavors persist better than the artificial.

4. The data obtained by chemical analysis can tell little with regard to the flavoring ability of the two general types of extracts.

5. If the Wichmann method is used to determine the lead number, an index may be obtained as to the genuineness of the vanilla extract. It should be remembered, however, that high lead numbers result from reinforcing extracts with excess vanillin, especially in the case of diluted extracts. The excess vanillin may be removed easily, however, thus nullifying its effect.

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INDEX TO VOLUME VII

ACID, Adulterated, as a possible source of error in testing milk by the Babcock method, 361

Acid, total, and volatile, A quantitative determination of, in cows' milk, 74

Acidity, A cheese factory test for critical degrees of, 83

Acidity, natural, in milk, The relation of, to composition and physical properties, 468

Advanced Registry news reports, 207

American Dairy Science Association, Annual meeting 1923, 107

American Dairy Science Association, Committee appointments for 1924, 309

American Dairy Science Association members for 1924, 413

Amino acid, in cows' milk, A quantitative determination of, 74

Ammonia, in cows' milk, A quantitative determination of, 74

Annual meeting, American Dairy Science Association, 1923, 107

Anti-scorbutic potency of milk, The influence of pasteurization and diet of the cow on the, 370

BACTERIAL content of ice cream, The, 330

Bacterial flora of the "Kingston Cheese," Further studies on the, 555

BARTLETT, JOHN W., MEAD, S. W., and REGAN, WM. M. A study of the factors affecting the growth of dairy heifers, 440

BENDIXEN, H. A. Review of foreign dairy literature, 407, 530

BIERMAN, H. R., and DOAN, F. J. A colorimetric picric acid method for determining lactose, 381

Birth weight and gestation of dairy animals, A study of, 222

Blindness and other diseases in children arising in consequence of deficient nutrition, 1

BLOCH, DR. C. E. Blindness and other diseases of children arising in consequence of deficient nutrition (Lack of fat soluble A factor), 1

BORST, W. F., PIERCE, H. B., COMBS, W. B. The use of true and imitation vanilla extracts in ice cream, 585

BRAMAN, WINFRED W., KRISS, MAX, FRIES, J. AUGUST. On the protein requirement of milk production, 11

BREED, R. S. Agricultural bacteriology in Italy, France and England as seen by a tourist, 205

BRINTNALL, EARLE. The official test in the south and the preliminary milking, 393

BRODY, SAMUEL, RAGSDALE, ARTHUR C., and TURNER, CHARLES W. The relation between age and fat production in dairy cows, 189

BRODY, SAMUEL, RAGSDALE, A. C., TURNER, C. W. The effect of gestation upon lactation in the dairy cow, 24

BRODY SAMUEL, RAGSDALE, A. C., TURNER, C. W. The rate of milk secretion as affected by an accumulation of milk in the mammary gland, 249

BURROUGHS, LILLIAN C. The disposal of dairy wastes, 503

Butter, carbonated, Facts about, 484

Butter, Hand-separator-cream, The improvement of flavor and keeping quality of, 460

Butter, The relation between yeasts and moulds and the keeping quality of, 357

Buttermilk, Condensed and powdered, for dairy calves, 213

CALCIUM, magnesium, phosphorus and sulfur in dairy cows fed high and low protein rations, The metabolism of, 58
Calves, dairy, Condensed and powdered buttermilk for, 213
Calves, dairy, Studies in the growth and nutrition of, IX. The addition of tomatoes to a milk ration, 94
Calves, dairy, Studies in the growth and nutrition of, X. Self-feeding a grain mixture to young calves, 160
Calves, Yeast as a supplementary feed for, 421
Carbonated butter, Facts about, 484
Cheese, Kingston, Further studies of the bacterial flora of the, 555
Cheese making, Clarification of milk for, 524
Children, Blindness and other diseases arising in consequence of deficient nutrition, 1
Clarification of milk for cheese making, 524
CLARK, R. S. The correlation between change in age and milk production of dairy cows under other than official testing conditions, 547
Color of cow's milk and its value, The, 147
Colorimetric picric acid method for determining lactose, A, 381
COMBS, W. B., BORST, W. F., PIERCE, H. B. The use of true and imitation vanilla extracts in ice cream, 585
COMBS, W. B., and MARTIN, W. H. The effect of milk plant operations on the amount of cream rising on milk, 197
COMBS, W. B., MARTIN, W. H., and HUGGLAR, N. A. Clarification of milk for cheese making, 524
Committee appointments by sections for 1924, American Dairy Science Association, 309
Computation of feeding standards, True protein versus crude protein as a basis for, 154
Condensed milk, Sweetened, II. A comparative study of methods for determining total solids, 497
COPELAND, LYNN, and FITCH, J. B. Variations in amount of milk and percent of fat in the milk from different quarters of the cow's udder, 169
COPELAND, LYNN, and OLSON, THOMAS M. The influence of pasteurization and diet of the cow on the antiscorbutic potency of the milk, 370
Correlation, The, between changes in age and milk production of dairy cows under other than official testing conditions, 547
Cow, dairy, The effect of gestation upon lactation in the, 24
Cows, Dairy, fed high and low protein rations, The metabolism of calcium, magnesium, phosphorus and sulfur in, 58
Cows, dairy, The correlation between age and milk production of, under other than official testing conditions, 547
Cows, dairy, The influence of the stage of lactation on the production of, 255
Cows, dairy, The relation between age and fat production in, 189
Cream rising on milk, The effect of milk plant operations on the amount of, 197
Cream tests, Reading the fat in, 131
Crude protein as a basis for computation of feeding standards, True protein versus, 154

DAHLE, C. D., PALMER, L. S. Some factors affecting the keeping quality of whole milk powders, 40
DAHLE, C. D., and PALMER, L. S. The effect of storage on the perox-

idase activity of whole milk powders, 141
Dairy literature, foreign, Review of, 306, 407, 530
Dairy literature, Review of, 210
Dairy notes, 205, 411
Dairy products, Vitamines in, 294
Dairy wastes, The disposal of, 503
Davidson, F. A. The effect of an incomplete removal of milk from the udder on the quantity and composition of the milk produced during the immediate subsequent milkings, 267
Doan, F. J. The color of cow's milk and its value, 147
Doan, F. J., and Bierman, H. R. A colorimetric picric acid method for determining lactose, 381
Drumm, G. M., Fitch, J. B., McGilliard, P. C. A study of the birth weight and gestation of dairy animals, 222
Duncombe, Eli. The influence of certain factors on the hydrogen ion concentration of milk, 86
Duncombe, Eli. The influence of certain factors on the hydrogen ion concentration of milk. II. Temperature changes, 245

Eaton, Sylvia M., and Zoller, Harper F. Sodium hypochlorite, the phenol coefficient and relative disinfecting power of, 318
Eckles, C. H., and Gullickson, T. W., Condensed and powdered buttermilk for dairy calves, 213
Eckles, C. H., Williams, V. M., Wilbur, J. W., Palmer, L. S., and Harshaw, H. M. Yeast as a supplementary feed for calves, 421
Enzyme peroxidase as found in milk, The effect of heat on, 234
Epple, W. F., and Spitzer, George. Reading the fat in cream tests, 131
Evenson, Oscar L., and Ferris, Leslie W. The viscosity of natural and remade milk, 174

Fat production in dairy cows, The relation between age and, 189
Fay, A. C., and Olson, N. E. The bacterial content of ice cream, 330
Feed consumption and milk secretion, A study of the relation between, 535
Ferris, Leslie W., and Evenson, Oscar L. The viscosity of natural and remade milk, 174
Fisher, Richard C., and Watts, Charles C. A comparative study of methods for determining total solids in ice cream, 576
Fisher, R. C. Review of foreign dairy literature, 306
Fisher, R. C., and Judkins, H. F. Measuring quality in ice cream, 31
Fisher, R. C., and Rice, Frank, E. Sweetened condensed milk. II. A comparative study of methods for determining total solids, 497
Fitch, J. B., and Copeland, Lynn. Variations in the amount of milk and percent of fat in the milk from different quarters of the cow's udder, 169
Fitch, J. B., McGilliard, P. C., and Drumm, G. M. A study of the birth weight and gestation of dairy animals, 222
Flavor and keeping quality of hand-separator-cream butter, The improvement, 460
Flora, bacterial, of the "Kingston Cheese," Further studies on the, 555
Forbes, E. B. True protein versus crude protein as a basis for computation of feeding standards, 154
Foreign dairy literature, review of, 306, 530
Frandsen, J. H. First American World's Dairy Congress a success, 138

FRIES, J. AUGUST, BRAMAN, WINFRED W., KRISS, MAX. On the protein requirement of milk production, 11

GAMBLE, J. A. Summary of minutes of meeting of southern division American Dairy Science Association, 1924, 403

Germany, Revival of dairy research in, 411

Gestation of dairy animals, and birth weight, A study of the, 222

Gestation, The effect of, upon lactation in the dairy cow, 24

GOWEN, JOHN W. Intrauterine development of the bovine fetus in relation to milk yield in Guernsey cattle, 311

Growth of dairy heifers, A study of the factors affecting the, 440

GULLICKSON, T. W., and ECKLES, C. H., Condensed and powdered buttermilk for dairy calves, 213

HAMMER, B. W. Associative action as a cause of yellow color in yeast colonies, 163

HARSHAW, H. M., and ECKLES, C. H. ET AL. Yeast as a supplementary feed for calves, 421

HASTINGS, E. G. A review of the fourth edition of the standard methods of milk analysis, 210

Heat, The effect of, on the enzyme peroxidase as found in milk, 234

Heifers, dairy, A study of the factors affecting the growth of, 440

HUGGLAR, N. A., COMBS, W. B., MARTIN, W. H. Clarification of milk for cheese making, 524

HUNT, GLENN A. Relation of milk production to the twinning tendency, 262

HUNZIKER, O. F. Facts about carbonated butter, 484

Hydrogen ion concentration of milk, The influence of certain factors on the, 86, 245

ICE cream, A comparative study of methods for determining total solids in, 576

Ice cream, Measuring quality in, 31

Ice cream, The bacterial content of, 330

Ice cream, The use of true and imitation vanilla extract in, 585

Intrauterine development of the bovine fetus in relation to milk yield in Guernsey cattle, 311

JUDKINS, H. F., FISHER, R. C. Measuring quality in ice cream, 31

KEEPING quality, and flavor, of hand-separator-cream butter, The improvement of, 460

KELLY, C. D. Further studies on the bacterial flora of "Kingston Cheese," 555

KRISS, MAX, FRIES, J. AUGUST, BRAMAN WINFRED W. On the protein requirement of milk production, 11

LACTATION in the dairy cow, The effect of gestation upon, 24

Lactation, The influence of the stage of, on the production of dairy cows, 255

Lactose, A colorimetric picric acid method for determining, 381

Lactose, in cows' milk, A quantitative determination of, 74

LINQUIST, HARRY G. Vitamines in dairy products, 294

LISK, HENRIETTA. A quantitative determination of the ammonia, amino nitrogen, lactose, total acid and volatile acid content of cows' milk, 74

McCANDLISH, ANDREW C. Studies in the growth and nutrition of dairy calves. IX. The addition of tomatoes to a milk ration, 94

McCANDLISH, ANDREW C. Studies in the growth and nutrition of dairy calves. X. Self-feeding a grain ration to young calves, 160

McCANDLISH, ANDREW C. The influence of the stage of lactation on the production of dairy cows, 255

McGILLIARD, P. C., DRUMM, G. M., FITCH, J. B. A study of the birth weight and gestation of dairy animals, 222

Magnesium, phosphorus, sulfur, calcium in dairy cows fed high and low protein rations, The metabolism of, 58

MARKLEY, ALTON L., and RICE, FRANK E. The relation of natural acidity in milk to composition and physical properties, 468

MARQUARDT, J. C. The revival of dairy research in Germany, 411

MARTIN, W. H., and COMBS, W. B. The effect of milk plant operations on the amount of cream rising on milk, 197

MARTIN, W. H., HUGGLAR, N. A., COMBS, W. B. Clarification of milk for cheese making, 524

MEAD, S. W., REGAN, WM. M., and BARTLETT, JOHN W. A study of factors affecting the growth of dairy heifers, 440

Measuring quality in ice cream, 31

Members, American Dairy Science Association for 1924, 413

Milk, Clarification of, for cheese making, 524

Milk, cows', A quantitative determination of the ammonia, amino nitrogen, lactose, total acid and volatile acid content of, 74

Milk, cows', The color of, and its value, 147

Milk, natural and remade, The viscosity of, 174

Milk plant operations, The effect of, on the amount of cream rising on milk, 197

Milk powders, whole, Some factors affecting the keeping quality of, 40

Milk powders, whole, The effect of storage on the peroxidase activity of, 141

Milk production, On the protein requirement of, 11

Milk production, The relation of, to the twinning tendency, 262

Milk secretion, A study of the relation between feed consumption and, 535

Milk secretion, The rate of, as affected by an accumulation of milk in the mammary gland, 249

Milk, Sweetened condensed, II. A comparative study of methods for determining total solids, 497

Milk, testing, by the Babcock method, Adulterated acid as a possible source of error in, 361

Milk, The effect of an incomplete removal of, from the udder on the quantity and composition of the milk produced during the immediate subsequent milkings, 267

Milk, The influence of certain factors on the hydrogen ion concentration of, 86

Milk, The influence of certain factors on the hydrogen ion concentration of, II. Temperature changes, 245

Milk, The relation of natural acidity in, to composition and physical properties, 468

Milk, Variations in the amount of, and percent of fat in, from different quarters of the cow's udder, 169

Milk yield in Guernsey cattle, Intrauterine development of the bovine fetus in relation to, 311

MONROE, C. F. The metabolism of calcium, magnesium, phosphorus, and sulfur in dairy cows fed high and low protein rations, 58

MORTENSEN, M. The improvement of flavor and keeping quality of hand-separator-cream butter, 460

Moulds, The relation between yeasts and, and the keeping quality of butter, 357

NOTES, Dairy, 205
Nutrition, Deficient, Blindness and other diseases in children arising in consequence of, 1

OFFICIAL test in the south and the preliminary milking, The, 393

OLSON, N. E., and FAY, A. C. The bacterial content of ice cream, 330

OLSON, THOMAS M., and COPELAND, LYNN. The influence of pasteurization and diet of the cow on the anti-scorbutic potency of the milk, 370

PALMER, L. S., and DAHLE, C. D. Some factors affecting the keeping quality of the whole milk powders, 40

PALMER, L. S., and DAHLE, C. D. The effect of storage on the peroxidase activity of whole milk powders, 141

PALMER, L. S., ECKLES, C. H., ET AL. Yeast as a supplementary feed for calves, 421

Pasteurization and diet of the cow, The influence of, on the anti-scorbutic potency of the milk, 370

PETERSEN, WM. E. Adulterated acid as a possible source of error in testing milk by the Babcock method, 361

Phenol coefficient and relative disinfecting power of sodium hypochlorite, 318

Phosphorus, sulfur, calcium, magnesium in dairy cows fed high and low protein rations, The metabolism of, 58

PIERCE, H. B., COMBS, W. B., and BORST, W. F. The use of true and imitation vanilla extracts in ice cream, 585

Powders, whole milk, Some factors affecting the keeping quality of, 40

Powders, whole milk, The effect of storage on the peroxidase acitivity of, 141

Preliminary milking, The official test in the south and the, 393

Protein requirement of milk production, 11

Protein, True, versus crude protein as a basis for computation of feeding standards, 154

QUALITY in ice cream, Measuring, 31

RAGSDALE, ARTHUR C., TURNER, CHARLES W., and BRODY, SAMUEL. The relation between age and fat production in dairy cows, 189

RAGSDALE, A. C., TURNER, C. W., and BRODY, SAMUEL. The effect of gestation upon lactation in the dairy cow, 24

RAGSDALE, A. C., TURNER, C. W., and BRODY, SAMUEL. The rate of milk secretion as affected by an accumulation of milk in the mammary gland, 249

REGAN, WM. M., MEAD, S. W., and BARTLETT, JOHN W. A study of the factors affecting the growth of dairy heifers, 440

Review of dairy literature, 210

RICE, FRANK E., and ALTON, MARKLEY, The relation of natural acidity in milk to composition and physical properties, 468

RICE, FRANK E., and FISHER, R. C. Sweetened condensed milk. II. A comparative study of methods for determining total solids, 497

SAMMIS, J. L., and SANTSCHI, FRED. A cheese factory test for critical degrees of acidity, 83

SANTSCHI, FRED, and SAMMIS, J. L. A cheese factory test for critical degrees of acidity, 83

Self-feeding a grain mixture to young calves, 160

SHUTT, DONALD BETHUNE. The relation between yeasts and moulds and the keeping quality of butter, 357

Sodium hypochlorite, The phenol coefficient and relative disinfecting power of, 318

Southern Division, American Dairy Science Association, Summary of minutes of meeting of, 403

SPITZER, GEORGE, and EPPLE, W. F. Reading the fat in cream tests, 131

SPITZER, GEORGE, and TAYLOR, M. C. The effect of heat on the activity of the enzyme peroxidase as found in milk, 234

Storage, The effect of, on the proxidase activity of whole milk powders, 141

Sulfur, calcium, magnesium, phosphorus, in dairy cows fed high and low protein rations, The metabolism of, 58

TAYLOR, M. C., and SPITZER, GEORGE. The effect of heat on the activity of the enzyme peroxidase as found in milk, 234

Test for critical degrees of acidity, A cheese factory, 83

Tomatoes, The addition of, to a milk ration for dairy calves, 94

Total solids in ice cream, A comparative study of methods for determining, 567

True protein versus crude protein as a basis for computation of feeding standards, 154

TURNER, CHARLES W., BRODY, SAMUEL, and RAGSDALE, ARTHUR C. The relation between age and fat production in dairy cows, 189

TURNER, C. W. A study of the relation between feed consumption and milk secretion, 535

TURNER, C. W., BRODY, SAMUEL, RAGSDALE, A. C. The effect of gestation upon lactation in the dairy cow, 24

TURNER, C. W., BRODY, SAMUEL, RAGSDALE, A. C. The rate of milk secretion as affected by an accumulation of milk in the mammary gland, 249

Twinning tendency, The relation of milk production to the, 262

UDDER, Cow's, Variations in the amount of milk and per cent of fat in the milk from different quarters of the, 169

VANILLA extracts in ice cream, The use of true and imitation, 585

Viscosity, The, of natural and remade milk, 174

Vitamines in dairy products, 294

WASTES, Dairy, The disposal of, 503

WATTS, CHARLES C., and FISHER, RICHARD C. A comparative study of methods for determining solids in ice cream, 576

WHITE, G. C. Advanced registry news reports, 207

WILBUR, J. W., and ECKLES, C. H., ET AL. Yeast as a supplementary feed for calves, 421

WILLIAMS, V. M., ECKLES, C. H., ET AL. Yeast as a supplementary feed for calves, 421

World's Dairy Congress a success, First American, 138

YEAST as a supplementary feed for calves, 421
Yeast colonies, Associative action as a cause of yellow color in, 163
Yeasts and moulds and the keeping quality of butter, The relation between, 357
Yellow color in yeast colonies, Associative action as a cause of, 163

ZOLLER, HARPER F., and EATON, SYLVIA M. Sodium hypochlorite, The phenol coefficient and relative disinfecting power of, 318

JOURNAL
OF
DAIRY SCIENCE

VOLUME VII
JANUARY, 1924, to NOVEMBER, 1924

1924
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BALTIMORE, U. S. A.

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CONTENTS

JANUARY. No. 1

Blindness and Other Diseases in Children Arising in Consequence of Deficient Nutrition (Lack of Fat Soluble A Factor). Dr. C. E. Bloch..... 1
On the Protein Requirement of Milk Production. J. August Fries, Winfred W. Braman and Max Kriss..... 11
The Effect of Gestation upon Lactation in the Dairy Cow. A. C. Ragsdale, C. W. Turner and Samuel Broady..... 24
Measuring Quality in Ice Cream, R. C. Fisher and H. F. Judkins..... 31
Some Factors Affecting the Keeping Quality of Whole Milk Powders. C. D. Dahle and L. S. Palmer..... 40
The Metabolism of Calcium, Magnesium, Phosphorus and Sulfur in Dairy Cows Fed High and Low Protein Rations. C. F. Monroe..... 58
A Quantitative Determination of the Ammonia, Amino Nitrogen, Lactose, Total Acid, and Volatile Acid Content of Cow's Milk. Henrietta Lisk.. 74
A Cheese Factory Test for Critical Degrees of Acidity. J. L. Sammis and Fred Santschi..... 83
The Influence of Certain Factors on the Hydrogen Ion Concentration of Milk. I. Eli Duncombe..... 86
Studies in the Growth and Nutrition of Dairy Calves. IX. The Addition of Tomatoes to a Milk Ration. Andrew C. McCandlish..... 94
Annual Meeting of the American Dairy Science Association..... 107

MARCH. No. 2

Reading the Fat in Cream Tests. George Spitzer and W. F. Epple..... 131
First American World's Dairy Congress a Success. J. H. Frandsen..... 138
The Effect of Storage on the Peroxidase Activity of Whole Milk Powders. C. D. Dahle and L. S. Palmer..... 141
The Color of Cow's Milk and Its Value. F. J. Doan..... 147
True Protein Versus Crude Protein as a Basis for Computation of Feeding Standards. E. B. Forbes..... 154
Studies in the Growth and Nutrition of Dairy Calves. X. Self-Feeding a Grain Mixture to Young Calves. Andrew C. McCandlish..... 160
Associative Action as a Cause of Yellow Color in Yeast Colonies. B. W. Hammer..... 163
Variations in Amount of Milk and Per Cent of Fat in the Milk from Different Quarters of the Cow's Udder. J. B. Fitch and Lynn Copeland..... 169
The Viscosity of Natural and Remade Milk. Oscar L. Evenson and Leslie W. Ferris..... 174
The Relation Between Age and Fat Production in Dairy Cows. Arthur C. Ragsdale, Charles W. Turner and Samuel Brody..... 189
The Effect of Milk Plant Operations on the Amount of Cream Rising on Milk. W. H. Martin and W. B. Combs..... 197

Dairy Notes 205
Review of Dairy Literature 210

MAY. No. 3

Condensed and Powdered Buttermilk for Dairy Calves. C. H. Eckles and T. W. Gullickson 213
A Study of the Birth Weight and Gestation of Dairy Animals. J. B. Fitch, P. C. McGilliard and G. M. Drumm 222
The Effect of Heat on the Activity of the Enzyme Peroxidase as Found in Milk. George Spitzer and M. C. Taylor 234
The Influence of Certain Factors on the Hydrogen Ion Concentration of Milk. II. Temperature Changes. Eli Duncombe 245
The Rate of Milk Secretion as Affected by an Accumulation of Milk in the Mammary Gland. A. C. Ragsdale, C. W. Turner and Samuel Brody 249
The Influence of the Stage of Lactation on the Production of Dairy Cows. Andrew C. McCandlish 255
Relation of Milk Production to the Twinning Tendency. Glenn A. Hunt 262
The Effect of an Incomplete Removal of Milk from the Udder on the Quantity and Composition of the Milk Produced During the Immediate Subsequent Milkings. F. A. Davidson 267
Vitamines in Dairy Products. Harry G. Lindquist 294
Review of Foreign Dairy Literature. R. C. Fisher 306
American Dairy Science Association 309

JULY. No. 4

Intrauterine Development of the Bovine Fetus in Relation to Milk Yield in Guernsey Cattle. John W. Gowen 311
The Phenol Coefficient and Relative Disinfecting Power of Sodium Hypochlorite. IV. Harper F. Zoller and Sylvia M. Eaton 318
The Bacterial Content of Ice Cream. A. C. Fay and N. E. Olson 330
The Relation Between Yeasts and Moulds and the Keeping Quality of Butter. Donald Bethune Shutt 357
Adulterated Acid as a Possible Source of Error in Testing Milk by the Babcock Method. Wm. E. Petersen 361
The Influence of Pasteurization and Diet of the Cow on the Anti-Scorbutic Potency of the Milk. Thomas M. Olson and Lynn Copeland 370
A Colorimetric Picric Acid Method for Determining Lactose. H. R. Bierman and F. J. Doan 381
The Official Test in the South and the Preliminary Milking. Earle Brintnall 393
Summary of Minutes of Meeting of Southern Division, American Dairy Science Association, Birmingham, Alabama, January 10, 1924. J. A. Gamble 403
Review of Foreign Dairy Literature. H. A. Bendixen 407
Dairy Notes 411
American Dairy Science Association Members for 1924 413

SEPTEMBER. No. 5

Yeast as a Supplementary Feed for Calves. C. H. Eckles, V. M. Williams and J. W. Wilbur. L. S. Palmer and H. M. Harshaw 421
A Study of the Factors Affecting the Growth of Dairy Heifers. S. W. Mead, Wm. M. Regan and John W. Bartlett 440
The Improvement of Flavor and Keeping Quality of Hand-Separator-Cream Butter. M. Mortensen 460
The Relation of Natural Acidity in Milk to Composition and Physical Properties. Frank E. Rice and Alton L. Markley 468
Facts About Carbonated Butter. O. F. Hunziker 484
Sweetened Condensed Milk. II. A Comparative Study of Methods for Determining Total Solids. R. C. Fisher and Frank E. Rice 497
The Disposal of Dairy Wastes. Lillian C. Burroughs 503
Clarification of Milk for Cheese Making. W. B. Combs, W. H. Martin and N. A. Hugglar 524
Review of Foreign Dairy Literature. H. A. Bendixen 530

NOVEMBER. No. 6

A Study of the Relation Between Feed Consumption and Milk Secretion. C. W. Turner 535
The Correlation Between Changes in Age and Milk Production of Dairy Cows Under Other Than Official Testing Conditions. R. S. Clark 547
Further Studies On the Bacterial Flora of the "Kingston Cheese." C. D. Kelly 555
A Comparative Study of Methods for Determining Total Solids in Ice Cream. Richard C. Fisher and Charles C. Watts 576
The Use of True and Imitation Vanilla Extracts in Ice Cream. H. B. Pierce, W. B. Combs and W. F. Borst 585
Index to Volume VII 591

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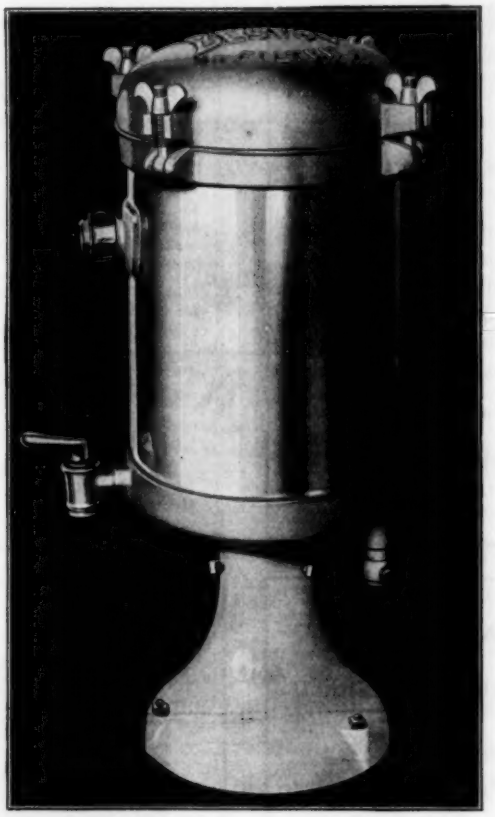

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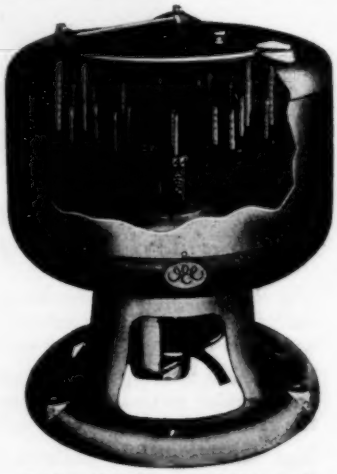

See Jour. Dairy Sci., Jan. 21, 1921, page 32

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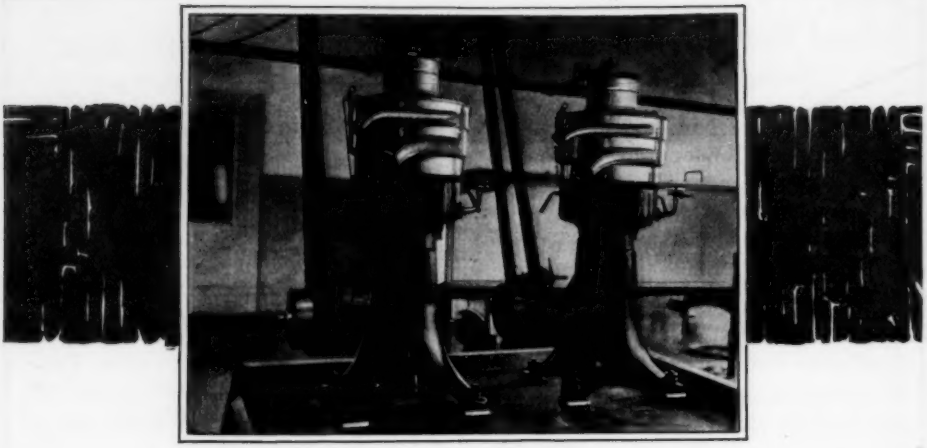

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